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SOME IMPORTANT PHASES OF THE DIABETIC PROBLEM WITH SPECIAL REFERENCE TO DIAGNOSIS*

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DIABETES mellitus is on the increase in the United States. Joslin[1] estimates that we have a million diabetics at the present time and insists that a campaign for its prevention holds the same promise and importance as preventive measures against tuberculosis, typhoid fever, and other diseases. The approach to the problem of prevention[2] lies along two lines, first, to detect the disease early, and second, to preclude its development in those known to be susceptible to it.

EARLY DIAGNOSIS

The early diagnosis[3, 4, 5] of diabetes in most cases is simpler than the early diagnosis of tuberculosis. It depends primarily on routine urine examinations by the general practitioner in all cases. In questionable cases the urine examinations should be supplemented by a fasting blood-sugar determination. Provided these two tests are made, few early diabetics will be overlooked. It is particularly important that obese individuals, patients with a suggestive hereditary or familial tendency to diabetes, patients convalescing from acute infectious diseases, and cases of chronic cholecystitis should have the foregoing examinations.

Life insurance examinations[6] have served to disclose a great number of silent glycosurias, and the plan of offering free urine examinations to policy

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holders will increase the number of early diabetics discovered. Likewise, specialists, including the dental surgeons, are demanding complete medical examinations before initiating operative procedures, so that an occasional case will be detected in this way.

I wish to direct attention to an important group which I am sure is common, but which offers certain difficulties in the diagnosis. This group is characterized by the presence of a transient glycosuria without a hyperglycemia or any of the usual clinical symptoms of diabetes, and cases of this group are frequently discovered in Life Insurance examinations and other routine medical surveys. The presence of glucose in the urine is found on the first examination, but on a repeated test, a few days later, glucose is absent. The consultant then sees the case, and sugar is again found. The important problem is, obviously, what is the significance of a transient glycosuria? The question can only be answered by studying the glucose tolerance,[7, 8, 9, 10] as determined by a blood-sugar curve following a dose of glucose. As previously mentioned, the fasting blood-sugar level is often normal, so that this determination alone affords no help. Interesting information can be obtained, however, by administering to the patient, on the fasting stomach, 100 grams of glucose dissolved in 400 c.c. of water to which the juice of an orange or lemon is added, and by plotting the curve of the blood-sugar levels at the half hour intervals up to two hours. In order to determine the fasting blood-sugar level and the presence, or absence, of sugar in the urine, it is important to collect the urine and the blood previous to the administration of the glucose solution. Besides the quantitative determinations of the glucose content of the blood at the half hour intervals, qualitative tests for the presence of sugar in the urine are also made.

THE SALIENT POINTS IN THE INTERPRETATION OF THE GLUCOSE TOLERANCE TEST

The individual who possesses the normal mechanism for glucose utilization will not develop a glycosuria on 100 grams of sugar at any period of this test. The amount of cane sugar which can be taken by most adults before sugar appears in the urine is from 200 to 250 grams.

Of greater importance, however, is the height to which the blood-sugar curve rises, the time required for it to reach its maximum height, and the time required for it to return to the normal fasting level. A maximum rise to a point over 0.17 per cent is abnormal, and a delay in return to the fasting level beyond the two hour interval usually indicates an impaired glucose utilization. Of the two criteria, the latter undoubtedly has greater significance. Disturbed utilization is also suggested by a curve which tends to rise to the maximum point slowly, since normally the rise to the maximum occurs promptly within the first half hour.

Unfortunately, the type of curve obtained is not pathognomonic of diabetes in all cases. Extensive studies[11, 12, 13] on the variations of the blood sugar have shown that other conditions than diabetes are at times associated with impaired glucose utilization and likewise abnormal blood-sugar curves. Chronic nephritis with hypertension, essential hypertension, such diseases of

the glands of internal secretion as hyperthyroidism and acromegaly, and gastric carcinoma are among the important well-known diseases that show abnormal curves. Nevertheless, with these conditions excluded, the glucose tolerance test offers evidence with regard to borderline diabetics which cannot be obtained by any other method.

It is my impression that it has not been sufficiently emphasized in literature that the estimation of the fasting blood sugar alone is not of diagnostic value, since in even a severe case of diabetes the fasting blood sugar may be normal, if the patient has been on a diet previous to the test. For this reason the glucose tolerance test has come to have an especial value in the study of the prediabetic, mild, and borderline cases.

A WIDER CONCEPTION OF THE SIGNIFICANCE OF GLYCOSURIA

Glycosuria, in itself, can no longer be regarded as a sign of a single disease. It appears more rational to think of glycosuria as a symptom like jaundice, angina, or headache; the cause of which must be ascertained in each case.

IDENTIFICATION OF A URINARY SUGAR

Any urine which shows a reducing substance with our copper reagents (i.e., Benedict's or Fehling's solutions) must be studied to determine which sugar, or sugars, is present. All reducing substances are not glucose, although glucose is by far the most common urinary sugar. Levulose, lactose, maltose, pentose, glycuronic acid and several other substances reduce copper solutions. It is well to remember, however, that glucose and levulose are the only sugars fermented by yeast. To differentiate glucose and levulose the polariscope is most useful, since glucose is dextrorotatory and levulose is levorotatory. Perhaps the most interesting characteristic of levulose is its failure to produce a hyperglycemia even after a 50 gram dose. It is believed that the absence of any appreciable elevation of the blood sugar after the administration of levulose by mouth may be attributed to the efficiency of the liver in removing this particular sugar from the blood and storing it as glycogen. In cases in which there is an inability on the part of the liver to deal with the ingested levulose, clinical evidence of hepatic disease is usually present; however, tests of this type do not permit of any exact quantitative estimate of the degree of impairment of the liver function.[14]

The presence of a substance which reduces copper solutions and at the same time does not ferment yeast, immediately puts it in the class of the unusual substances. Lactosuria[15, 16] of pregnancy and the lactating state is perhaps the most confusing and frequently encountered urinary sugar besides glucose. It can be differentiated from glucose by Mathews' method,[17] the essential basis of which is the fermentation of urine with a large amount of yeast. The glucose is quickly fermented away and its reducing action destroyed, while lactose remains unaffected in the filtrate. The identification of the other urinary sugars and reducing substances requires the application of special chemical tests which are described in books on clinical diagnostic methods.[18]

THE RENAL THRESHOLD

The term renal threshold has come into common usage since the advent of routine blood-sugar determinations. It means the concentration of the sugar in the blood at which point sugar first appears in the urine. It is now thought that this point is a blood-sugar concentration of about 0.17 per cent; however, this is a variable level not only in different individuals but also probably in the same individual at different times. The renal threshold value has come into practical importance chiefly because of its variations both above and below the normal average level of 0.17 per cent.

RENAL GLYCOSURIA

An interesting familial condition, first described by Klemperer[19] about thirty years ago, emphasized a lowering of the renal threshold so that an anomalous condition came about, in which glycosuria existed with a normal glycemia. This was first called renal diabetes but is now usually referred to as renal glycosuria. Subsequent studies have shown that it is characterized by four cardinal findings; namely, first, specimens of urine and blood collected simultaneously must demonstrate a glycosuria with a normal or depressed glycemia; second, there must be no symptoms of clinical diabetes mellitus present, i.e., loss of weight, polyuria, polydipsia, etc.; third, glycosuria must not parallel the carbohydrate content of the diet, i.e., increase of carbohydrate in the diet should not increase the amount of sugar in the urine; fourth, the patient must not later develop into a true case of diabetes mellitus. This latter stipulation Joslin has always stressed, since he feels that unquestionable instances of renal glycosuria are uncommon and that many of the reported cases have not been followed for a sufficiently long period to be sure that true diabetes has not eventually developed. This attitude of conservatism in the diagnosis of renal glycosuria, I feel, has much to commend it, particularly until more has been learned about the mechanism controlling the renal threshold for glucose and until the ultimate outcome of the established cases of this condition has been followed for a longer time.

ELEVATION OF THE RENAL THRESHOLD

The opposite condition to renal glycosuria with reference to the renal threshold, namely, a hyperglycemia without glycosuria, ocurs frequently in patients who have been treated with insulin. Major and Davis[20] have recently reported a short series of such cases. Advanced renal sclerosis is also occasionally accompanied by this condition. At the present moment there is no accepted explanation for the elevation of the renal threshold in these cases. Allen[21] has considered this point in connection with some of his dog experiments. He is strongly of the opinion that this raising of the threshold is a disadvantage to the animal, since the internal secretion of the pancreas is being overworked whenever the level of the blood sugar is considerably raised. With this fact in mind, it seems rational to try to keep the blood sugar at the normal level at all times in diabetes by diet restriction and proper

insulin dosage, including both the total amount of insulin given and the spacing of the doses.

NONDIABETIC GLYCOSURIAS ASSOCIATED WITH AN ELEVATED BLOOD SUGAR

It is gradually becoming apparent that there are some clearly defined groups of cases which present glycosuria with hyperglycemia, but which are not true instances of diabetes. I shall try to confine this discussion to four such groups.

The first comprises those examples of excessive ingestion of sugar by a normal individual. This is the so-called alimentary glycosuria[22] and is frequently found in young persons following an excessive intake of candy.

The next group of importance we may term the neurogenic[23, 24] type. Occasionally it is found that a student following an examination passes sugar in the urine. This emotional glycosuria is believed to be the result of an overproduction of glucose in the sense that, because of the nervous stimulation of the liver, there is a rapid conversion of glycogen to glucose, with the accumulation of the latter in the blood stream. Glycosuria follows this elevation of the blood sugar.

Other forms of the neurogenic glycosuria of a similar pathogenesis are seen after fracture of the skull, intracranial hemorrhage, and during toxic states of acute infections. The association of unconsciousness and glycosuria has an especial clinical significance, since it suggests at once the diagnosis of diabetic coma.

A third type of nondiabetic glycosuria with hyperglycemia occurs during such asphyxia as is commonly produced by carbon monoxide or the anesthetic drugs and is probably due to difficulty in oxidation.

The fourth group is a large and poorly understood one which is associated with disturbance in the glands of internal secretion. It is accepted by physiologists that several of these glands influence the metabolism of carbohydrates. They recognize that glycosuria is a common feature of hyperthyroidism[25, 26, 27] and of exophthalmic goiter, and that associated with this there is usually marked hyperglycemia. The glucose tolerance test shows an abnormal elevation of the blood sugar and in some instances a delay in the return to the fasting level. The diminished carbohydrate tolerance of hyperthyroidism is, in fact, one of the most constant of the abnormalities of carbohydrate metabolism met with in endocrine diseases. Respiratory experiments, however, demonstrate that there is no decrease in the patient's ability to oxidize glucose but that probably there is a defect in the storage mechanism which fails to handle the excessive mobilization of glucose, a diametrically opposite state than that of diabetes.

The glycosuria of pituitary disturbances[28] is another endocrine manifestation and is, supposedly, caused by a hypersecretion of the posterior lobe of the hypophysis. The importance of the pituitary body in controlling the concentration of sugar in the blood is shown by the fact that it is impossible to produce the characteristic convulsions of insulin hypoglycemia in animals in which the cerebral hemispheres have been removed but the pituitary body

left intact. If the pituitary body is also removed, adequate injections of insulin are followed by the typical hypoglycemic reaction. A considerable proportion of the obese patients show a curve suggestive of a mild degree of impairment of carbohydrate tolerance,[29] and it probably is advisable to regard such cases as potential diabetics; at the same time it must be recognized that in some patients of this type the hyperglycemia and obesity may be the expression of a pituitary disorder, often dating from pregnancy.

Epinephrin administered subcutaneously causes a discharge of glycogen from the liver into the blood stream and is frequently associated with a hyperglycemia and at times with a glycosuria. The hyperglycemia and glycosuria that follow puncture of Claude Bernard's "diabetic center" in the floor of the fourth ventricle are believed to be due to a stimulation of the splanchnics and excessive secretion of epinephrin.

GLYCOSURIA WITH THE HYPERGLYCEMIA OF TRUE PANCREATIC DIABETES

I will now pass to a brief consideration of true pancreatic diabetes with glycosuria and hyperglycemia. In this condition the abnormality lies in the oxidation of glucose rather than in its mobilization or ingestion. Diabetes mellitus is due to a disturbance in the function of the Islands of Langerhans of the pancreas, the primary inciting cause of which, in most instances, is still unknown, although infection and degenerative arteriolar processes probably play an important part.

In spite of the fact that this disease is usually comparatively simple to recognize clinically, difficulty in diagnosis is encountered in three groups of cases: (1) Asymptomatic glycosuria; (2) patients complaining of the complications of diabetes only; (3) patients first seen in coma.

THE ASYMPTOMATIC GROUP

The characteristics of this group and the modern method of diagnosis have already been discussed under the heading of early diagnosis. I wish to reemphasize the extreme importance of this type of case and to insist that the physician must be on the alert to the problem presented. Dietetic restriction and insulin therapy are, obviously, contraindicated in nonpancreatic glycosurias. On the other hand, to dismiss an individual casually, with the assurance that his glycosuria is of no significance, may eventually throw him into the class of uncontrolled diabetes. Each case should be studied carefully and an opinion reached on the merits of the findings. Naunyn's teaching, that all glycosurias should be regarded as diabetic until they are proved otherwise, still holds true.

PATIENTS COMPLAINING OF ONE OF THE COMPLICATIONS OF DIABETES AS THEIR PRESENTING SYMPTOM

This group is easy to diagnose provided diabetes is thought of as the underlying cause of an apparently unrelated condition. For example, I recently discovered a well-marked glycosuria in a patient who had been treated by a competent ophthalmologist for months for a neuroretinitis. Apparently the diabetic etiology of this eye condition had never been considered.

Similarly I have recently seen an elderly gentleman who had been suffering for months with a generalized pruritis and an eczematous skin eruption on the dorsum of the feet and around the ankles. His family physician had treated him by the application of local remedies for several months without satisfactory results. Finally the patient consulted a neurologist, who promptly found a large amount of sugar in the urine. Such examples could be multiplied. Several instances of diabetes treated locally by the urologist for sexual impotence have come to my notice. In all instances, the difficulty has been the failure to consider the possibility of the underlying cause for a particular symptom; in other words, the attention has been focussed too closely on the complication, whereas the cause has been overlooked.

PATIENTS FIRST SEEN IN COMA

Unconsciousness in a patient with glycosuria demands use of the physician's utmost diagnostic skill, in order that he may avoid hasty and harmful injection of insulin, to adjust the treatment to both the diabetes and probable complications, and to protect the rights of the patient's family. Coma is the state of unconsciousness which follows diabetic acidosis. Clinically, it is characterized by "air hunger" and, chemically, by an excess in the blood and urine of the end-products of faulty fat metabolism. The onset of coma is usually gradual and is associated with anorexia, headache, abdominal cramps, and vomiting. The precipitating cause is usually an acute infection, a nervous upset, or an unusual exertion, but the real cause is always dietary error. The physical findings show a patient with deep and rapid respiration, dry skin, cold extremities, subnormal temperature, rapid pulse, low blood pressure, soft eyeballs, flushed cheeks, and at times an odor of acetone on the breath.

Joslin[30] has recently emphasized some of the special points of clinical importance in diabetic coma. He points out that the fat diabetic can go into coma, but it takes gross mismanagement to produce it. In his series of 48 coma cases only one exceeded 140 pounds at the time of coma. The leucocytosis frequently found is usually secondary to gastric hemorrhage, provided infection can be excluded. The preliminary examination should be made for the purpose of seeking an endogenous cause for the coma by searching for localized infections, pneumonia, and such intraabdominal complications as a ruptured appendix, kidney complications, or paranephric abscess. The blood pressure is an important determination and often offers a valuable index of the patient's condition and progress. It tends to run comparatively low in most cases, the systolic pressure frequently being found under 100 mm.

The essential laboratory findings vary, but a catheterized specimen of urine will show sugar in moderate amount, whereas the blood sugar is relatively higher and often falls between 0.40 per cent and 0.80 per cent. The urine gives a maximum red-black reaction to ferric chloride for the presence of diacetic acid. If the facilities for the determination of the carbon dioxide combining power of the blood plasma are available, it is usually found below 15 per cent by volume; when above 30 per cent by volume, it betrays either a complication or the previous administration of alkali. Joslin has shown that the human organism is tolerant to an extraordinary degree to extreme altera-

tions in the carbon dioxide content of the blood, and he "wonders whether acidosis and alkalosis ever reach such a stage in the human body as of themselves to cause death. Are not the deaths which we associate with these variations in the blood carbon dioxide really due to other factors in the overwhelming majority of instances? Such factors are intercurrent infections, septicemias, heart failure from exhaustion, a dilated stomach, or lack of body fluids."

Other causes for the clinical picture simulating coma are numerous and include such things as uremia, apoplexy, meningitis, trauma, hypoglycemia due to inanition or overdosage with insulin, narcotic poisoning, and brain tumor.

SUMMARY

To control the increasing prevalence of diabetes in this country it is necessary to detect the early cases and to preclude the development of this disease in those groups of individuals known to be susceptible to it.

Special attention is called to a large group of early or so-called latent cases in which the proper study of the individual furnishes important data for the future management of the patient. In this group the glucose tolerance test supplies significant information not obtainable by any other means. The estimation of the fasting blood sugar alone is not always of diagnostic value, since even in a severe case of diabetes the fasting blood sugar may be normal if the patient has been on a diet previous to the test.

Glycosuria, in itself, can no longer be regarded as a sign of a single disease. It appears more rational to think of glycosuria as a symptom such as jaundice, angina, or headache, the cause of which must be ascertained in each case.

The diagnosis of renal glycosuria should not be arrived at hastily, since unquestionable instances of this condition are uncommon and few cases have been followed for a sufficient period to be certain of the final outcome.

An elevation of the renal threshold, so that hyperglycemia exists without glycosuria, occurs occasionally in diabetics who have been treated with insulin. Present information would indicate that a constant elevation of the threshold is a disadvantage to the organism because of the excessive work continuously thrown upon the internal secretion of the pancreas.

The four important groups of nondiabetic glycosurias associated with an elevated blood sugar content are the alimentary, neurogenic, toxic, and the endocrine.

The endocrine group at the present time represents a large and poorly understood one which is associated with a disturbance in the function of the glands of internal secretion. Perhaps the most outstanding example is the diminished carbohydrate tolerance of hyperthyroidism as shown by the presence of glycosuria and an abnormal blood-sugar curve. The glycosurias accompanying pituitary and adrenal disturbances need further experimental study.

True pancreatic diabetes offers especial difficulty in the diagnosis primarily in the three following groups of cases: (1) Asymptomatic glycosuria; (2) patients complaining of the complications of diabetes only; (3) patients first seen in coma.

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THE SIGNIFICANCE OF CHANGES IN THE COMPOSITION OF THE BLOOD AND URINE AFTER THE INGESTION OF GLUCOSE*

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HARROP and Benedict[1] have observed a fall in the inorganic phosphorus of the blood serum simultaneously with the rise in blood sugar after the ingestion of large doses of glucose. In animals, when the glucose was combined with insulin administration, these authors noted a fall in the inorganic phosphorus of the blood, a rise in the organic phosphorus of the muscles, and a diminished phosphate excretion in the urine. These findings indicate a shift in the mobile phosphate stores of the body during the process of active carbohydrate assimilation. Blatherwick, Bell, and Hill,[2] however, were able to find no consistent change in the inorganic phosphorus of the blood or urine after ingestion of glucose. In some instances an increase was noted, in some a decrease, and in others no change. But after the administration of insulin a marked fall of the inorganic phosphorus of the blood serum and a drop of the phosphorus excretion in the urine were consistently reported. Briggs, Koechig, Doisy, and Weber[3] have shown a decrease in the concentration of glucose, inorganic phosphorus and potassium of the blood of normal animals with a parallel rise in the lactic acid under the influence of insulin. Fiske[4] has studied the influence of carbohydrate ingestion on phosphoric acid excretion in the urine. He states that carbohydrate combustion is accompanied by retention of phosphate, which is later excreted at an exaggerated rate. All of the data recorded in these and similar studies lend additional support to the belief that a combination of hexose with phosphoric acid is an intermediate step in the metabolism of carbohydrate.

With this hypothesis in mind, an attempt was made to get more exact information concerning the metabolism of carbohydrate in glucose tolerance studies in cases of hyperthyroidism, by making simultaneous determinations of the inorganic phosphorous of the blood and urine along with the sugar. The procedure utilized in glucose tolerance studies was that outlined by Killian.[5] The adopted method of handling these cases is as follows: after a fourteen or fifteen hour fast the patients are received in the respiration laboratory for a determination of the basal metabolism.† Thence they come to the chemical laboratory where they receive a standard breakfast, consisting of one egg (boiled), one cup of coffee containing two teaspoonfuls of cream (40 per cent) and two slices of white bread. This meal contains 11.0 grams of fat, 13.7 grams of protein and 37.0 grams of carbohydrate, repre-

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†The basal metabolic rates were determined by Dr. Bailey and to him I am indebted for the privilege of using these data.

senting a total value of 311.7 calories. This breakfast is sufficient to break the fast; the relief of which we find essential for the comfort of the patient. It also makes it possible to study the metabolism of all cases with a definite relation to the time of food intake. Two hours after the breakfast, the bladder is emptied by voluntary voiding, and the patient takes, by mouth, 100 c.c. of water. At the end of one hour after taking the water, a specimen of blood is obtained and a specimen of urine. These two specimens are the controls and represent the composition of the blood and urine three hours after the standard meal. Thereupon glucose is given by mouth, 1.75 grams per kilogram of body weight and in a 50 per cent aqueous solution. During this time the patient is maintained at rest. It has been found that cerelose is a very satisfactory sugar for carbohydrate tolerance tests. Following the ingestion of glucose, specimens of blood and urine are obtained at hourly intervals for two or three hours. The Folin-Wu[6] method was employed for the blood-sugar determinations, and Benedict and Osterberg's[7] method for the urine sugar. Where the sugar of the urine exceeded 0.5 per cent, check determinations were made by the Benedict volumetric method. For the phosphorus Benedict's technic[8] was followed. In all cases care was taken to prevent hemolysis.

In Table I are recorded the data obtained on 9 cases of hyperthyroidism. It will be seen that, although in no instance does the blood obtained at the end of the control hour show a hyperglycemia, the sugar output in the urine during this period is abnormally high. Killian has reported a normal excretion of about 30 mg. for the control hour. The ingestion of the glucose produces, in all but one case, a hyperglycemia persisting for two hours. In Cases 1 and 2 the urine sugar rises markedly in response to changes in the blood sugar, but in the other instances the effect upon the urine sugar is less marked, and in Subject No. 5 the urine sugar remains unchanged. It is not possible to demonstrate any parallelism between the elevation of the basal metabolic rate and the character of the blood-sugar curve after the glucose. This is to be expected. The basal metabolism is a measure of the energy produced in oxidation reactions within the body in a postabsorptive state. The conversion of glucose, however, into glycogen through the intermediate stage of hexose phosphate and lactic acid evolves only 5 per cent of the total caloric value of the glucose. There is noted no consistent change in the inorganic phosphorus of the blood. Cases 7 and 8 show slight increases, the others a slight decrease. The maximum decrease is usually found after the blood sugar has begun its return to normal, but this decrease in the blood inorganic phosphorus is insignificant when compared with the changes in the blood sugar. With the exception of Cases 8 and 9, in all instances the ingested glucose produced a marked increase in the urine phosphorus, which, however, bore no relation to the amount of sugar excreted. In Patients 8 and 9 the phosphorus was diminished in the urine.

Similar observations have been made on 13 cases representing normal and miscellaneous pathologic conditions, without, however, any detectable

TABLE I

CHANGES IN SUGAR AND INORGANIC PHOSPHORUS OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE IN HYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD		URINE		BASAL METABOLIC RATE PER CENT	REMARKS
				SUGAR	INORGANIC PHOSPHORUS	SUGAR	INORGANIC PHOSPHORUS		
				MG.	MG.	MG.	MG.		
1. A. W.	40	M	Control	106	5.7	76	10.1	+108	Toxic exophthalmic goiter
			1st	246	5.6	744	17.6		
			2nd	224	5.4	1110	58.3		
			3rd	91	5.8	2642	45.3		
2. L. P.	19	F	Control	93	4.3	312	20.0	+ 95	Exophthalmic goiter
			1st	205	3.8	2627	18.6		
			2nd	150	3.7	3814	22.1		
			3rd	75	4.4	316	28.1		
3. L. B.	32	M	Control	95	4.8	74	56.2	+ 72	Exophthalmic goiter
			1st	192	4.3	786	85.2		
			2nd	150	3.7	363	80.0		
			3rd	69	4.3	105	46.8		
4. J. P.	38	F	Control	78	3.7	69	19.4	+ 51	Exophthalmic goiter
			1st	211	3.6	145	35.0		
			2nd	148	3.4	462	45.0		
			3rd	75	3.9	74	26.6		
5. M. K.	37	F	Control	102	4.0	32	31.2	+ 12	Small goiter with hyperthyroidism
			1st	205	4.1	28	41.3		
			2nd	156	3.9	31	38.8		
			3rd	115	4.1	28	29.7		
6. A. L.	45	F	Control	105	5.5	64	34.3	+ 71	Exophthalmic goiter
			1st	184	4.9	100	47.0		
			2nd	174	5.4	—	—		
7. E. L.	28	F	Control	115	4.5	19	14.4	+ 56	Exophthalmic goiter
			1st	130	5.3	123	66.2		
			2nd	100	5.3	70	74.9		
8. W. L.	23	M	Control	114	3.8	66	29.6	+ 33	Hyperthyroidism
			1st	227	4.2	81	24.6		
			2nd	203	3.2	1770	22.0		
9. B. W.	20	F	Control	98	4.6	72	39.6	+ 32	Exophthalmic goiter
			1st	275	4.5	510	18.0		
			2nd	204	4.3	505	14.0		

change in the thyroid function. The results obtained are recorded in Table II. In this group the basal metabolic rates ranged from 11 per cent below to 14 per cent above the average normal. Four subjects were normals, and they present changes in the sugar of the blood and urine after the glucose, within the limits considered by Killian as typical of the normal. The remaining cases, however, show a mildly lowered tolerance for carbohydrate, but there is no evident relationship between the blood- and urine-sugar curves and the variations in heat production as measured by the basal metabolism. The effect produced upon the inorganic phosphorus of the blood and the urine of the normals by the glucose is analogous in all respects to that observed in cases showing a moderate decrease in carbohydrate tolerance, and in cases of hyperthyroidism presenting a much greater lowering of glucose assimilation. In one instance the blood phosphorus rose from 5.0 to 5.6 in two hours, paralleled by a decrease in the urinary phosphorus from 29 to

TABLE II

CHANGES IN SUGAR AND INORGANIC PHOSPHORUS OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE IN NONHYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD		URINE		BASAL METABOLIC RATE PER CENT	REMARKS
				SUGAR MG.	INORGANIC PHOSPHORUS MG.	SUGAR MG.	INORGANIC PHOSPHORUS MG.		
1. F. C.	16	F	Control	84	5.7	22	17.6	+ 1	Normal
			1st	133	5.2	31	30.8		
			2nd	100	5.6	14	22.8		
2. G. D.	12	M	Control	106	5.0	34	29.0	−11	Normal
			1st	144	5.2	17	12.5		
			2nd	119	5.6	16	19.6		
3. B. F.	9	M	Control	110	5.2	21	28.5	+ 2	Normal
			1st	144	5.2	18	33.0		
			2nd	138	5.0	23	25.0		
4. H. N.	13	M	Control	80	5.0	29	19.8	− 2	
			1st	214	4.9	33	28.5		
			2nd	147	5.0	53	51.2		
5. J. B.	15	M	Control	110	4.7	50	23.4	+14	Speech defect
			1st	161	4.7	59	25.3		
			2nd	165	4.5	44	25.1		
6. J. V.	17	M	Control	100	5.1	38	17.1	+ 6	Mental defective
			1st	200	4.9	83	32.7		
			2nd	163	4.7	157	37.8		
7. G. L.	20	M	Control	79	4.5	73	29.9	+12	Normal
			1st	152	4.3	—	—		
			2nd	107	4.6	198	59.7		
8. C. L.	9	M	Control	89	5.3	50	42.0	− 3	Emotional instability
			1st	195	5.1	20	31.4		
			2nd	144	5.2	32	42.2		
9. A. G.	29	F	Control	106	4.0	77	8.0	+ 1	Obesity
			1st	195	3.9	67	21.3		
			2nd	147	4.0	61	20.7		
10. B. K.	23	F	Control	87	4.7	41	12.8	+ 1	Epilepsy
			1st	208	4.1	708	30.5		
			2nd	130	4.1	412	15.0		
11. E. O.	20	M	Control	99	3.4	64	28.3	− 5	Psychoneurosis
			1st	176	3.1	57	35.2		
			2nd	144	3.6	54	37.5		
12. S. P.	25	M	Control	86	4.4	30	26.8	− 9	
			1st	202	3.6	37	40.3		
			2nd	147	3.7	46	40.7		
13. H. M.	17	M	Control	116	5.0	28	20.0	+13	Psychoneurosis
			1st	125	5.0	39	42.0		
			2nd	135	4.8	49	49.2		
			3rd	86	4.7	32	38.4		

19.6 mg. per hour. In all other instances a fall in the inorganic phosphorus of the blood is accompanied by an increase in the urine, and this rise in the urinary phosphorus steadily increases to reach its maximum at the end of the first hour after the glucose and then declines. A comparison of the data in Tables I and II demonstrates that the changes in the inorganic phosphorus of the blood or of the urine bear no quantitative relation to the rate at which the glucose is removed from the blood. Harrop and Benedict[1] observed the

TABLE III

CHANGES IN SUGAR AND CHLORIDE OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE IN HYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD SUGAR MG.	BLOOD Cl AS NaCl MG.	URINE SUGAR MG.	URINE Cl AS NaCl MG.	BASAL METABOLIC RATE PER CENT	REMARKS
1. E. P.	19	F	Control	93	478	312	620	+95	Exophthalmic goiter
			1st	205	462	2627	460		
			2nd	150	486	3814	560		
			3rd	75	478	316	170		
2. J. P.	38	F	Control	78	495	69	440	+51	Exophthalmic goiter
			1st	211	495	145	280		
			2nd	148	495	462	240		
			3rd	75	495	74	620		
3. E. W.	29	F	Control	90	554	51	828	+46	Adenomatous goiter with hyperthyroidism
			1st	107	548	52	348		
			2nd	123	511	84	402		
			3rd	124	508	60	187		
4. L. B.	32	M	Control	95	500	74	423	+72	Exophthalmic goiter
			1st	192	463	786	440		
			2nd	150	500	363	352		
			3rd	69	488	105	215		
5. G. G.	33	F	Control	83	500	92	902	+60	Exophthalmic goiter
			1st	258	495	801	192		
			2nd	214	495	750	280		
			3rd	68	495	56	200		
6. M. G.	32	F	Control	125	503	44	392	+38	Hyperthyroidism
			1st	238	503	238	200		
			2nd	187	511	350	273		
7. F. L.	50	F	Control	95	511	85	600	+11	Hyperthyroidism
			1st	246	511	333	110		
			2nd	242	511	1125	207		
8. M. P.	17	F	Control	130	525	60	608	− 4	Hyperthyroidism
			1st	170	513	157	203		
			2nd	79	513	273	270		

maximum fall in blood phosphorus about two hours after the glucose; this is about the same as the decrease seen in the cases reported above. This drop in blood inorganic phosphorus is minute in comparison with the sugar assimilated. Since it is accompanied, moreover, by a simultaneous rise in the urine phosphorus, it does not appear plausible to explain the decrease in the blood as due to demands made upon the blood phosphorus for the formation of hexose phosphate. It is seen from the tables that the maximum rise in the urinary phosphorus appears after the blood sugar has begun its return to normal. A possible explanation of this increased output of phosphorus suggests itself. Phosphorus may be drawn from the tissues through the medium of the blood for the formation of the hexose phosphate. With the storage of the hexose as glycogen, the phosphorus is released and excreted in the urine as inorganic phosphorus.

It seemed possible that this depressing effect might be extended to some of the other inorganic constituents of the blood in addition to the phosphates. Changes in the chlorides of the blood and urine produced by glucose taken by mouth have been studied in 8 subjects with hyperthyroidism and in 9 without hyperthyroidism. The results obtained are presented in Tables III

TABLE IV

CHANGES IN SUGAR AND CHLORIDE OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE IN NONHYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD		URINE		BASAL METABOLIC RATE PER CENT	REMARKS
				SUGAR MG.	Cl AS NaCl MG.	SUGAR MG.	Cl AS NaCl MG.		
1. D. C.	20	M	Control	82	475	40	1250	+ 6	
			1st	86	475	51	1150		
			2nd	84	475	58	1290		
			3rd	64	475	42	990		
2. F. C.	30	M	Control	77	500	216	1316	+18	Mitral stenosis
			1st	130	500	48	150		
			2nd	126	500	105	209		
			3rd	106	500	51	137		
3. K. P.	21	M	Control	134	513	545	32	−35	Nephrosis
			1st	147	525	823	34		(Cholesterol = 0.286)
			2nd	135	500	895	40		
4. A. M.	31	F	Control	110	528	63	832	+ 2	Obesity (Thyroid therapy for eight months)
			1st	148	512	38	410		
			2nd	92	495	52	519		
5. L. D.	55	F	Control	99	511	48	884	+ 3	Hysteria
			1st	205	503	49	557		
			2nd	150	495	44	441		
6. E. H.	15	M	Control	93	488	54	813	− 1	Epilepsy
			1st	166	475	53	312		
			2nd	165	471	32	62		
7. G. W.	26	F	Control	88	495	58	760	+15	Encephalitis Lethargica
			1st	140	478	67	756		
			2nd	120	495	55	720		
8. H. M.	17	M	Control	116	513	28	730	+13	Psychoneurosis
			1st	125	486	39	274		
			2nd	135	488	49	456		
			3rd	86	500	32	322		

and IV. Whitehorn's method was utilized for the determination of the chlorides of the whole blood, and the Volhard-Harvey procedure for the urine chlorides. In a few instances the blood chlorides remained unchanged after the glucose administration (Nos. 2 and 7 of Table III, and 1 and 2 of Table IV) and in but one case was there an increase (No. 6 of Table III). The remaining cases showed a decrease in the blood chlorides with a return to the control concentration in about three hours. The minimum figure obtained for the chlorides does not always correspond in time with the maximum blood sugar. Where the chlorides return to their control concentration, this return lags behind the drop in the blood sugar. There is, moreover, no apparent quantitative relation between the drop in the blood chlorides and the height of the blood-sugar curve. In Case 5 (Table III) the rise in blood sugar is 175 mg. per 100 c.c., but the drop in the chlorides is but 5 mg. per 100 c.c. On the other hand, in No. 3 (Table III) the blood chlorides decrease 46 mg., but the sugar rises only 34 mg. When, however, the rate of assimilation of the glucose is gauged by a consideration of the blood-sugar curve in association with the urine-sugar curve, there appears to be some dependence of the fall of the blood chlorides upon the amount of the glucose utilized. An accurate estimate of the glucose assimilated cannot be made

from these data, but the observation seems warranted that the fall in the blood chlorides appears greater in those cases in which the glucose ingested did not produce a persistent or marked hyperglycemia accompanied by a glycosuria.

The decrease in the chlorides of the blood is associated with a markedly diminished output in the urine. In the urine the drop in the chlorides amounts to 60 to 90 per cent of the original concentration, whereas the maximum decrease observed for blood was but 8 per cent of the control figure. Case 3 (Table IV) was one of nephrosis with a pronounced general anasarca. Here it is observed that the chloride output for the control hour was but 32 mg., less than 5 per cent of that observed for the other subjects. There is noted, moreover, but a slight increase in the urine chlorides after the glucose ingestion. Herrick[9] has demonstrated what he calls a "reciprocal relationship" between the chlorides and the glucose of the blood after the ingestion of 100 grams of glucose. This author states three possible factors producing this depression of the blood chlorides after the glucose intake: there may be an increased elimination in the urine, a dilution of the blood, or a shift of the chlorides into the other tissues of the body.

An increased elimination of urine chlorides cannot account for their fall in the blood, since in all of the cases studied the chlorides of the urine were greatly diminished. As a check upon the possibility of a dilution of the blood after the glucose by mouth, hemoglobin determinations were made upon specimens before and after glucose. The procedure used in these determinations was that described by Myers.[14] It is evident from the data in Table V that the ingestion of the glucose, as employed in the experiments described, produced very little change in the hemoglobin concentration of the blood. The maximum decrease was but 0.8 grams per 100 c.c. amounting to about 4 per cent of the original concentration. In the majority of the cases, however, the drop in hemoglobin was insignificant. There appears to be no appreciable dilution of the blood resulting from the glucose. This factor, then, may be eliminated as a possible cause of the depression of the blood chlorides. Since the determinations of blood chlorides recorded in the tables have been made on whole blood, the observed changes in their concentration in the blood cannot be explained by an interchange between the plasma and the erythrocytes. The shift from the blood to other tissues as described by Herrick appears to be the only tenable hypothesis to account for the phenomenon. In a subsequent communication further data bearing upon this topic will be presented.

The influence of glucose taken by mouth upon the level of the nonprotein nitrogen of the blood and of the total nitrogen of the urine was studied in 5 subjects. References to Table VI shows that following the ingestion of the glucose there is but a slight change in the nonprotein nitrogen of the blood during the two or three hours of observation. Subject 5 showed no change, Subjects 2 and 3 showed an increase of 3.4 and 6 mg. respectively; and 1 and 4 showed a decrease of 3.2 and 2 mg. There was no uniform change and no evident relation of the variation of the nonprotein

TABLE V

CHANGES IN SUGAR AND HEMOGLOBIN OF BLOOD, VOLUME AND SUGAR OF URINE AFTER INGESTION OF GLUCOSE IN HYPERTHYROIDISM

CASE	AGE	SEX	TEST HOUR	BLOOD		URINE		BASAL METABOLIC RATE PER CENT	REMARKS
				SUGAR MG. PER 100 C.C.	HEMOGLOBIN GRAMS PER 100 C.C.	VOLUME C.C.	SUGAR MG. PER HOUR		
1. A. W.	40	M	Control	106	13.9	84	76	+108	Toxic exophthalmic goiter
			1st	246	13.6	31	744		
			2nd	224	13.3	137	1110		
			3rd	91	13.3	104	2642		
2. J. P.	32	F	Control	78	12.7	57	69	+ 51	Exophthalmic goiter
			1st	211	12.2	44	145		
			2nd	148	11.6	100	462		
			3rd	75	12.3	275	74		
3. L. B.	32	M	Control	96	12.8	45	74	+ 72	Exophthalmic goiter
			1st	192	13.4	55	786		
			2nd	150	12.9	40	363		
			3rd	69	12.9	25	105		
4. M. G.	32	F	Control	125	14.2	35	44	+ 38	Hyperthyroidism
			1st	238	13.4	25	238		
			2nd	187	13.4	35	350		
5. S. G.	40	F	Control	84	16.0	30	19	+ 19	Exophthalmic goiter
			1st	250	15.8	60	257		
			2nd	77	15.2	52	240		
6. H. M.	17	M	Control	116	15.6	166	28	+ 13	Psychoneurosis
			1st	125	15.5	28	39		
			2nd	135	15.2	34	49		
			3rd	86	15.4	23	32		
7. M. P.	17	F	Control	130	15.2	80	60	− 4	Hyperthyroidism
			1st	170	14.6	22	157		
			2nd	79	15.0	30	273		

nitrogen to the character of the blood-sugar curve. Similar changes are seen in the total nitrogen of the urine. Cases 1 and 4 gave an increased output of urine nitrogen, and it is of interest to note that in both these individuals the increased urinary nitrogen is accompanied by a fall in the nonprotein nitrogen of the blood. On the other hand, in Cases 2 and 3 the urine nitrogen diminishes with a subsequent return towards normal, and corresponding to this decrease in the urine nitrogen, there is observed an increase in the nonprotein nitrogen of the blood with a subsequent decrease. No. 5, however, shows an increased output of urine nitrogen with no change in the nonprotein nitrogen of the blood which was unusually low. Although there is no direct proportion between the nitrogen excreted in the urine and the volume of the urine, in general it may be said that an increase in the volume of urine excreted entails a rise in the output of nitrogen and that a decrease in the volume of urine produces a diminished elimination of nitrogen. The changes in nitrogen of the blood and urine after glucose appear to be due to the diuresis produced by the glucose.

Since the literature on carbohydrate metabolism presents an abundance of evidence that lactic acid is an essential intermediate product in the storage of carbohydrate as glycogen, or in its oxidation to CO_2 and H_2O, it seemed

TABLE VI

CHANGES IN SUGAR AND NITROGEN OF BLOOD AND URINE AFTER INGESTION OF GLUCOSE

CASE	AGE	SEX	TEST HOUR	BLOOD		URINE			BASAL METABOLIC RATE PER CENT	REMARKS
				SUGAR MG. PER 100 C.C.	N. P. N. MG. PER 100 C.C.	SUGAR MG. PER HOUR	TOTAL N. MG. PER HOUR	VOLS.		
1. M. B.	26	F	Control	115	30.0	32	263	21	+6	Hyperthyroidism
			1st	288	30.0	1700	541	170		
			2nd	242	26.8	720	291	48		
2. L. M.	27	F	Control	100	28.9	20	550	204	+4	Hyperthyroidism
			1st	227	32.3	320	436	64		(very excitable)
			2nd	103	32.1	99	491	74		
3. F. F.	15	F	Control	91	24.0	80	650	260	−2	(Headaches)
			1st	205	30.0	144	500	75		
			2nd	182	24.0	1292	636	140		
4. M. W.	17	F	Control	83	30.0	43	357	50	+1	Epilepsy
			1st	174	29.0	75	392	40		
			2nd	106	28.0	97	566	170		
5. M. N.	40	F	Control	98	21.1	40	157	33	−9	Arteriosclerosis
			1st	98	21.0	35	206	43		Gastric neoplasm
			2nd	135	21.0	37	210	32		
			3rd	108	21.0	20	97	17		

possible that a study of the changes in the lactic acid content of the blood, following the ingestion of glucose along with the blood- and urine-sugar curves, might give valuable information concerning the rate of glycogenesis. On this point, however, the opinions of some investigators are at variance. Mendel, Engel, and Goldscheider[10] reported no changes in the lactic acid of the blood in two individuals after taking 50 grams of glucose by mouth. Collago and Lewicki[11] found in the blood of normal individuals and diabetics similar amounts of lactic acid; they also found a rise in the blood lactic acid in both groups of cases after the ingestion of sucrose. Nishimura[12] has found an increase in the lactic acid of the blood after taking 1.75 grams of glucose per kilogram of body weight.

In a group of 10 cases, including 6 hyperthyroids, 2 psychoneuroses, and 2 normals, the variations in the lactic acid of the blood have been followed with the blood and urine sugar after the taking of glucose. During the entire period of observation the subjects were maintained at rest, lying upon a couch.

Clausen's method[13] was employed for the lactic acid determinations. The blood was drawn without stasis and analyzed immediately. Under such conditions Nishimura has noted the lactic acid content of normal bloods within the limits of 11.7 to 18.0 with an average of 15 mg. per 100 c.c. It will be seen in Table VII that the cases of hyperthyroidism show a slightly higher figure for the lactic acid in the control blood, ranging from 13.7 to 30.6 mg., the remaining cases present figures from 10.5 to 16.9 mg. per 100 c.c. Following the intake of the glucose, all cases show a rise in the lactic acid, paralleling the hyperglycemia. Cases 3, 5, 6, and 7 show a lowered tolerance for carbohydrate. The glucose in these subjects produces a hyperglycemia persisting for more than two hours, accompanied by a markedly increased output of sugar in the urine. In these instances the average maximum rise in the

TABLE VII

CHANGES IN SUGAR AND LACTIC ACID OF BLOOD AFTER INGESTION OF GLUCOSE

CASE	AGE	SEX	TEST HOUR	BLOOD SUGAR MG.	BLOOD LACTIC ACID MG.	URINE SUGAR MG.	BASAL METABOLIC RATE PER CENT	REMARKS
1. H. R.		M	Control	98	10.5			
			1st	134	11.7			
			2nd	119	36.9			
2. H. K.	30	M	Control	97	11.7			
			1st	179	17.5			
			2nd	122	15.7			
3. E. P.	19	F	Control	93	17.8	312	+95	Exophthalmic goiter
			1st	205	23.4	2627		
			2nd	150	22.5	3814		
			3rd	75	11.3	316		
4. E. W.	29	F	Control	90	16.2	51	+46	Adenomatous goiter with hyperthyroidism
			1st	107	23.8	52		
			2nd	123	27.4	84		
			3rd	124	18.4	60		
5. L. B.	32	M	Control	95	20.8	74	+72	Exophthalmic goiter
			1st	192	24.3	786		
			2nd	150	22.9	363		
			3rd	69	19.8	105		
6. M. G.	32	F	Control	125	30.6	44	+36	Hyperthyroidism
			1st	238	34.2	238		
			2nd	187	32.8	350		
7. A. P.	32	F	Control	100	17.1	63	+10	Hyperthyroidism
			1st	300	20.7	1160		
			2nd	220	18.0	1482		
8. F. K.	14	M	Control	105	13.7	163	+22	Hyperthyroidism
			1st	183	15.9	53		
			2nd	118	14.4	116		
9. F. S.	26	M	Control	99	16.9	63	+ 4	Dementia precox
			1st	183	20.3	3203		
			2nd	79	18.0	121		
10. P. M.	23	M	Control	96	13.9	35	+15	Neurasthenia
			1st	126	15.3	52		
			2nd	132	21.6	29		

lactic acid of the blood after the glucose is from 3.5 to 4.7 mg., but in the remaining cases with fairly normal tolerance for carbohydrate, this rise runs from 5.8 to 26.4 mg. In these cases studied it appears that individuals with lowered tolerance for carbohydrate present less change in the lactic acid of the blood after glucose than cases with normal or increased tolerance. The data reported in Table VII do not warrant general conclusions, but an intensive study of this phase of the problem is under way at present, and the results will be presented in the near future.

SUMMARY

The ingestion by mouth of 1.75 grams of glucose per kilogram of body weight in 50 per cent solution, three hours after a standard meal, produced in the majority of subjects a slight drop in the inorganic phosphorus of the blood with a markedly increased output in the urine. In a few instances the blood phosphorus was increased. In but 3 of the 22 subjects studied was

the inorganic phosphorus of the urine diminished. These changes in the blood and urine phosphorus after glucose were similar for hyperthyroid and non-hyperthyroid subjects. The increased output of urine phosphorus may be due to the release of the phosphate combined as hexose phosphate when the hexose is converted into glycogen.

Changes in blood and urine chlorides after glucose were studied in 16 individuals. In 4, the blood chlorides remained unchanged, in 2 an increase and in 10 a decrease were found. In but one individual was an increased urine chloride noted; the remainder showed a strikingly diminished excretion of chlorides. Changes in the blood chlorides cannot be due to a dilution of the blood. The intake of the glucose produced no appreciable change in the hemoglobin content of the blood in 7 cases reported. A shift of the chlorides from the blood to other tissues appears to be the only tenable explanation of the changes noted.

Following the administration of the glucose in some instances a fall of the nonprotein nitrogen of the blood was accompanied by an increased output of urine nitrogen. In other cases the blood nonprotein nitrogen rose with a fall in the urine nitrogen.

The ingestion of glucose produced a rise in the lactic acid of the blood. This rise was greater in individuals with normal tolerance for carbohydrate than in those with a diminished tolerance.

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URIC ACID STUDIES*

I. Comparison of the Direct and the Isolation Methods of Uric Acid Determination in Blood Filtrates and a Modification of Folin's Method

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THE reaction of uric acid with phosphomolybdic reagents, as well as with phosphotungstic reagents, which results in the formation of a blue color, described by Frabot and by Riegeler,[1] was adapted by Folin and Denis to the determination of uric acid in the blood and other body fluids in 1913,[2] and since then many modifications of the reagent have been proposed. The nature of the compound to which the blue coloration is due is still unknown. The reagents are not specific for uric acid. It is known that the characteristic blue color may also be obtained by the action of the reagents on phenol, polyhydroxi-phenols, certain amino-acids and adrenalin, all of which substances may be present in the protein-free filtrate. This applies equally to Folin's phosphotungstic acid reagent[3] and to Benedict's arsenophosphotungstic acid reagent.[4] Notwithstanding this fact the so-called direct method, which omits any attempt at the separation of the uric acid from these substances, is often employed. The direct method of determination has certain advantages. It shortens the procedure, and the use of sodium carbonate to bring about the necessary alkalinity following the solution of the precipitated urate as uric acid is unnecessary. Furthermore a possible loss of the uric acid present, due to incomplete precipitation, is precluded.

The main difficulty encountered in the present methods is due to the precipitation of a hard, white, crystalline substance, which often occurs before colorimetric readings are obtained. This difficulty is not overcome by the omission of the preliminary isolation, but apparently the frequency with which precipitation occurs is increased, probably because of the greater concentration of tungstates and sulphates in the reaction mixture. If the precipitation occurs shortly after the reagent has been added, it may be removed by centrifugation without loss in color of the supernatant fluid, provided the precipitation was complete before centrifuging. It may happen that after spinning and obtaining a clear, supernatant fluid, the process of precipitation is still continued. A second separation consumes too much time, and the color is likely to fade before readings can be obtained. The same difficulty is encountered when the precipitation occurs just before the solutions are ready for reading. The phosphotungstic salts present are in a supersaturated solution, and the slightest dust or solid particle of any kind will

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initiate precipitation. Since many of the solutions employed, such as the cyanide, the carbonate, as well as the reagent, attack glass, the presence of minute solid particles can only be avoided by keeping them in paraffined containers and by frequent filtering. While the addition of a small amount of lithium sulphate as advocated by Folin,[3] as well as strict observance of the precautions mentioned above, will reduce the frequency with which precipitation occurs, the danger is not avoided. The modification of the method introduced in our experiments and reported in this communication increases the stability of the final solution, so that precipitation practically never occurs, although the precautions as to paraffined containers and frequent filtering are not observed. Furthermore the color developed is more intense and is stable for twenty-four hours.

It has been stated that precipitation of uric acid in the protein-free filtrate by silver lactate in lactic acid solution accomplishes the seemingly impossible, since silver lactate does not react to form insoluble silver urate with uric acid present in the concentrations such as maintain in the blood filtrate, from aqueous solution.[4] The presence of other radicles which form highly insoluble silver salts aids in the precipitation of the silver urate. We have tested the precipitation capacity of the silver lactate reagent on known solutions of uric acid in sulphuric-tungstic acid solution of concentration such as prevails in the blood filtrate and using our modification of Folin's method obtained a recovery varying between 95 per cent and 100 per cent. The average of eight experiments was 98 per cent. Practically the same results were obtained when sodium chloride in N/10 HCl was used as the solvent for the precipitated urate, the recovery varying between 92 per cent and 100 per cent. By using basic zinc carbonate for the preliminary isolation we were unable to recover appreciable amounts of uric acid present in the tungstic acid solution. Our modification of Folin's uric acid method was also tested by running comparative analyses on blood filtrates. The results varied only within the experimental error of the method and proved that the methods are comparable.

Silver lactate in common with other silver salts has a tendency to decompose. Many of the so-called C. P. preparations have a distinct grayish tinge. If this is pronounced, and if there is considerable insoluble material present when making the 5 per cent solution in 5 per cent lactic acid, it is best to determine the silver content of the filtered solution. Since the solution also has a distinct tendency to reduction it must be kept in small amber glass bottles, or the bottles may be painted on the outside with optical black. The addition of NaOH, to partially neutralize the lactic acid, as advocated by Folin,[3] should be avoided, since it increases the tendency to reduction. The presence of reduced silver must be guarded against in the determinations, as the reduced silver forms a double salt with the NaCN which also gives a blue color with the reagent. This source of error has been pointed out by Morris and McLeod,[5] who for this reason prefer basic zinc carbonate as the isolating agent. If during the analysis, however, the reaction mixtures are kept from the direct light and not allowed to stand too long between centrifuging, there

is no danger of formation of free silver. This is readily accomplished by keeping the centrifuge tubes in which the reaction is carried out in the metal containers and allowing them to remain in the closed centrifuge for the five minutes which ought to elapse before the first spinning. Our experiments with uric acid added to the blood filtrates, as well as with uric acid solutions in sulpho-tungstic acid, point to the silver lactate as the more efficient precipitant. When the uric acid was added either to whole blood or to plasma prior to the precipitation of the proteins, the recovery of the added uric acid lay between 53 to 72 per cent. That this loss is due to adsorption of the uric acid on the precipitated proteins is borne out by the fact that the loss in concentration was the same whether the determinations were made on the

TABLE I

RECOVERY OF URIC ACID ADDED TO WHOLE BLOOD

CONC. OF URIC ACID BEFORE ADDITION MG. %		ADDED URIC ACID IN MG. %	DETERMINED CONC. OF URIC ACID MG. %		URIC ACID RECOVERED IN PER CENT OF AMOUNT ADDED		% URIC ACID RECOVERED. CORRECTED FOR DRY VOLUME	
Direct	Isolated		Direct	Isolated	Direct	Isolated	Direct	Isolated
4.3	2.8	2.5	6.1	4.5	72%	68%	48%	46%
—	2.5	4.0	—	5.7	—	80%	—	56%
4.8	3.2	3.0	7.0	5.5	73%	77%	51%	54%
2.2	1.8	6.0	6.7	6.5	75%	78%	56%	59%
6.0	4.0	1.5	7.2	5.1	80%	73%	56%	52%
4.5	2.7	5.0	8.5	6.7	80%	80%	53%	53%
					Average recovery		53%	53%

TABLE II

RECOVERY OF URIC ACID ADDED TO BLOOD PLASMA

CONC. OF URIC ACID BEFORE ADDITION MG. %		ADDED URIC ACID IN MG. %	DETERMINED CONC. OF URIC ACID MG. %		URIC ACID RECOVERED IN PER CENT OF AMOUNT ADDED		% URIC ACID RECOVERED. CORRECTED FOR DRY VOLUME	
Direct	Isolated		Direct	Isolated	Direct	Isolated	Direct	Isolated
4.5	2.6	2.5	6.3	4.5	72%	76%	60%	63%
5.00	3.0	3.0	7.5	5.4	83%	80%	69%	67%
2.2	1.5	2.0	4.1	3.5	95%	100%	79%	85%
6.0	4.0	4.0	9.1	7.0	77%	75%	64%	62%
2.5	2.0	2.0	4.5	4.0	100%	100%	83%	83%
					Average recovery		71%	72%

filtrate directly or by preliminary isolation. (Tables I and II.) In plasma the concentration of the adsorbant (the precipitated proteins) is far less than in whole blood, and one would therefore expect a better recovery. The amount of uric acid recovered when it is added to plasma as against that when the uric acid is added to whole blood is far greater than the analyses show. The last two columns on Tables I and II show the corrections for the dry volume, i.e., the volume occupied by the precipitated proteins. As has been pointed out,[6] this error is in direct proportion to the dry volume. Twenty c.c. of the protein-free filtrate obtained from whole blood with an average cell volume of 45 per cent will contain the soluble substances present in approximately 3 c.c. of whole blood, whereas the same amount of filtrate obtained from the plasma of the same blood will contain the soluble sub-

TABLE III

URIC ACID DETERMINATIONS ON BLOOD FILTRATES DIRECT AND BY THE PRELIMINARY ISOLATION METHOD

	URIC ACID DETERMINED WITH FOLIN'S REAGENT MG. %			URIC ACID DETERMINED WITH BENEDICT'S REAGENT MG. %			
	On Filtrates Direct	Preliminary Isolation	Difference	On Filtrates Direct	Preliminary Isolation	Difference	
No. 1163	5.00	1.45	3.55	—	—	—	Pernicious vomiting of pregnancy.
A. Jo.	6.10	2.70	3.40	6.80	2.70	4.10	" " " "
No. 245	4.80	2.27	2.53	—	—	—	" " " "
C. Oat.	9.60	4.24	5.36	11.20	2.20	6.80	" " " "
M. Mh.	7.00	2.20	4.80	7.80	2.20	5.60	" " " "
Vesz.	14.60	6.65	7.95	—	—	—	" " *not* pregnant.
No. 4219	7.22	5.80	1.42	—	—	—	Toxemia of pregnancy. Uricacidemia.
No. 4219	4.97	2.66	2.31	—	—	—	" " " "
No. 4344	6.75	4.40	2.35	7.10	4.60	2.50	" " " "
Bro.	5.43	3.67	1.76	5.65	3.55	2.10	" " " "
Bly.	6.94	3.44	3.50	—	—	—	" " " "
Men.	4.65	3.20	1.45	5.00	3.30	1.70	" " " "
Wea.	6.98	4.32	2.66	7.20	4.32	2.88	" " " "
No. 4289	5.10	3.56	1.54	5.20	3.50	1.70	" " " "
Bro.	7.60	5.20	2.40	7.95	5.20	2.75	Toxemia of pregnancy with N. retention.
"	9.23	7.30	1.90	—	—	—	" " " " " "
Mc G.	8.00	5.90	2.10	8.55	6.00	2.55	" " " " " "
" "	8.60	5.26	3.34	—	—	—	" " " " " "
" "	10.40	6.25	4.15	—	—	—	" " " " " "
Benj.	12.10	7.45	4.65	—	—	—	" " " " " "
"	10.40	5.40	5.00	11.00	5.55	5.45	" " " " " "
Dur.	10.30	8.1	2.20	—	—	—	Toxemia of pregnancy. Eclampsia.
No. 3586	8.16	5.26	2.90	—	—	—	" " " "
No. 4282	9.00	8.10	0.90	9.60	7.85	1.75	" " " "
No. 4282	9.95	6.65	3.30	—	—	—	" " " "
Ag.	9.80	8.75	1.05	—	—	—	" " " "
Fr.	9.10	7.40	1.70	10.00	7.40	2.60	" " " "

TABLE III—CONT'D

	URIC ACID DETERMINED WITH FOLIN'S REAGENT MG. %			URIC ACID DETERMINED WITH BENEDICT'S REAGENT MG. %			
	On Filtrates Direct	Preliminary Isolation	Difference	On Filtrates Direct	Preliminary Isolation	Difference	
Nix.	13.30	9.10	4.20	—	—	—	Toxemia of pregnancy. Eclampsia
Leve.	7.45	4.15	3.30	—	—	—	Nephritis.
No. 1175	4.00	3.00	1.00	4.22	3.10	1.12	"
Mu.	3.80	2.20	1.60	—	—	—	"
O'D.	7.85	4.70	3.15	8.25	4.50	3.75	"
Re.	6.05	4.00	2.05	—	—	—	"
Sem.	8.00	3.24	4.76	—	—	—	Hypertension.
No. 4576	6.70	3.22	3.48	—	—	—	"
No. 2276	6.25	3.58	2.67	—	—	—	"
No. 5075	5.40	2.36	3.04	—	—	—	"
Dwy.	9.50	3.10	6.40	—	—	—	Diabetes mellitus.
Dieh.	7.41	5.26	2.15	—	—	—	" " and nephritis.
Schw.	9.08	2.84	6.24	—	—	—	" " and arteriosclerosis.
Mil.	5.60	2.60	3.00	—	—	—	Diabetes mellitus.
No. 21003	5.00	3.18	1.82	5.30	3.00	2.30	Arthritis.
Ka.	5.00	2.70	2.30	—	—	—	"
Fat.	9.10	4.45	4.65	10.00	4.75	5.25	Gout. Indicanuria.
"	9.50	5.75	4.75	—	—	—	
No. 1235	7.40	4.35	3.05	—	—	—	Arthritis.
Acc.	7.15	4.92	2.23	—	—	—	Gout.
Ros.	6.40	2.88	3.52	—	—	—	Nephrosis.
Eich.	5.00	2.41	2.59	—	—	—	"
No. 63923	6.96	5.20	1.76	—	—	—	Pyoderma.
Rus.	10.00	5.40	4.60	—	—	—	Treat. syph.
Mona.	7.70	2.60	5.10	—	—	—	Ulcers.
Wei.	5.00	2.52	2.48	—	—	—	Urticaria.

stances present in approximately 2.4 c.c. of plasma. That this error is partially compensated for by the adsorption of the soluble substances present in the filtrate on the precipitate must be conceded, but the extent of adsorption is indeterminate. Correcting for the dry volume the average recovery of the uric acid added to whole blood was 53 per cent, while for the uric acid added to plasma it was 72 per cent.

The results of comparative uric acid analyses, using Folin's as well as Benedict's reagents and carrying out the determinations on the filtrate direct as well as by preliminary isolation, are given in Table III. The results point to the desirability of employing a preliminary isolation method. The analyses given are representative of the results obtained in various pathologic conditions. The difference in results between the two methods (direct and preliminary isolation) while of varying degree is constantly present. Experiments on a small group of "normals" confirmed the findings of Bulmer, Eagles, and Hunter[7] of the existence of a distinctive difference between the two methods. The analyses carried out on the filtrate direct are invariably higher, irrespective of the reagent used. The differences vary from 12 per cent to 120 per cent.

The method used for the determination of the uric acid in the filtrate obtained by Folin's precipitation method was as follows:

Ten, fifteen, or twenty cubic centimeters of the filtrate are pipetted into a clean and dry 50 c.c. pyrex centrifuge tube with constricted end. Five cubic centimeters of the silver lactate reagent (5 per cent silver lactate in 5 per cent lactic acid) are added. The mixture is allowed to stand for five minutes *in the dark* and next centrifuged for five to seven minutes at top speed (2,500 R.P.M.). After carefully decanting the supernatant fluid, 3 c.c. of 10 per cent lithium chloride solution are added, and the mixture is stirred with a very fine glass rod. Eight to ten c.c. of distilled water are next added, the mixture well stirred and the rod finally washed down with about 2 c.c. of water. The tube is centrifuged *immediately* for eight to ten minutes at top speed and the clear supernatant fluid transferred to a 25 c.c. volumetric flask. From a burette 2 c.c. of recently filtered 15 per cent NaCN solution are added. One c.c. of the reagent is now added with shaking and the color allowed to develop for twenty to thirty minutes. The solutions may be made to mark immediately after adding the reagent or just before reading in the colorimeter.

Simultaneously the standard is set up in a 50 c.c. flask, using 1 or 2 c.c. of a standard uric acid solution, containing 0.1 mg. uric acid per c.c., in Benedict's and Hitchcock's phosphate solution.[8] Water to about 30 c.c. is added, next 6 c.c. of 10 per cent lithium chloride solution, 4 c.c. of the NaCN solution and 2 c.c. of the reagent.

The standard uric acid solution in phosphate solution, containing 100 mg. per liter, will keep indefinitely if certain precautions are observed. The standard must be kept in small amber glass containers, or containers painted on the outside with optical black, of approximately 200 c.c. capacity. Prior to use the containers are set up with cleaning fluid overnight, washed with

tap water followed by a thorough rinsing with distilled water, and next steamed for fifteen to twenty minutes. The apparatus described by Findlay for the steaming of conductivity vessels is simple and well adapted to this purpose.[9] The bottles after filling and adding 5 c.c. of redistilled toluol are corked, paraffined, and kept in the ice box. In this way the standard of the bottle in use will be exhausted within a short enough time to eliminate all danger of decomposition due to exposure to the atmosphere. We have found that uric acid solution prepared and kept in this manner will not lose in concentration even though kept for a period of eighteen months. Needless to say the uric acid must be carefully recrystallized before use and the phosphate solution boiled and filtered.

Upon the addition of silver lactate to the blood filtrate, silver chloride, silver tungstate, silver phosphates, as well as silver urate, are precipitated. The use of lithium chloride as solvent for the silver urate in this mixture, as well as a precipitant for the excess silver present, has distinct advantages over the use of NaCl in dilute HCl. Lithium urate is far more soluble than uric acid, and none of the other silver salts present are as readily dissociated by the lithium chloride as by the N/10 HCl. The solution obtained is practically neutral, and the use of the carbonate or of the cyanide in too large a concentration in order to obtain the alkalinity necessary for the final reaction is also avoided. The use of the carbonate, as well as of the cyanide, in large concentration increases the tendency to precipitation. The use of the cyanide in greater concentration than employed in this method is also contraindicated for the reason that the cyanide itself gives a color with the Folin or the Benedict reagents.

Two uric acid standards were set up, using identical quantities with the exception of the cyanide which was doubled in one of the solutions. It was found that using the Folin reagent and doubling the cyanide causes an error in the final reading of 48 per cent, while with the Benedict reagent the error was 25 per cent.

The method has also been adapted for the determination of uric acid in the urine.

Five c.c. of albumin-free urine are pipetted into a 25 c.c. volumetric flask; water to approximately 20 c.c. is added, and the solution rendered neutral to litmus by the addition of 30 per cent NaOH or 25 per cent acetic acid, drop by drop. One to two drops generally suffice. The solution is then made to volume. If the urine contains a large amount of phosphates, they are first dissolved by the addition of a few drops of acetic acid. Urines containing deposits of urates are warmed, diluted one to one with distilled water to prevent redeposit, and 10 c.c. of the cooled mixture pipetted into the 25 c.c. flask. The albumin, if any, is removed from the slightly acidified urine by coagulation and subsequent centrifugation, care being taken to avoid loss by evaporation. Two c.c. of the diluted urine (equivalent to 0.4 c.c. of urine) are employed in the estimation. Five c.c. of silver lactate reagent are added to the 2 c.c. of the dilute urine in a 25 c.c. or 50 c.c. pyrex centrifuge tube. The reaction mixture is allowed to stand *in the dark* for five minutes and then

spun at high speed for five minutes. Before decanting the clear supernatant fluid, it is best to ascertain that precipitation has been complete by adding a drop of the silver lactate solution to the supernatant fluid. After decanting the clear supernatant fluid, 6 c.c. of LiCl solution are added, and the mixture is thoroughly stirred with a fine glass rod. When the precipitate is equally divided and all particles broken up, about 12 to 15 c.c. of water are added, the whole stirred well, and the rod washed down with about 2 c.c. of water. The tubes are spun immediately at high speed for ten minutes. The clear supernatant fluid is decanted into a 50 c.c. volumetric flask, water added to about 30 c.c.; next, 4 c.c. of the 15 per cent NaCN, followed by the reagent, added slowly with constant shaking. Allow to stand for at least twenty minutes and read against a 2 c.c. standard set up in the usual manner. Such a standard will suffice for a uric acid concentration ranging from 35 to 70 mg. per cent. For weaker concentrations a 1 c.c. standard must be used, for stronger concentrations a 3 c.c. standard. Using a 2 c.c. standard the calculation reduces to $\frac{\text{St.} \times 50}{\text{Rdg. X}}$ = mg. per cent. Since the color is stable for twenty-four hours and fully developed within twenty minutes, no difficulty is experienced if a stronger standard is found necessary. It is best to dilute 1:10 urines which contain a large amount of uric acid and to use 2 c.c. of this dilute urine for the determination, thus employing 0.2 c.c. instead of 0.4 c.c. of urine.

The results of the analyses given in Tables III and IV prompted us to carry out experiments to determine to what extent the presence of phenol, polyhydroxi-phenols, or certain amino-acids will increase the blue color of the reaction. For this purpose known amounts of phenol, of resorcinol, or of tyrosine, were added to aliquot parts of blood or plasma. The uric acid determinations were also carried out on control bloods or plasmas by the direct as well as by the isolation method. The results of these examinations are given in Table IV.

The procedure employed was briefly as follows: The blood or plasma was divided into aliquot parts of 5 or 8 c.c. On one such part the analyses were carried out in the usual manner, thus serving as control. To one of the other parts phenol in water solution was added, to another tyrosine in N/HCl solution, and to the last resorcinol in water solution. The volume of the addition, never exceeding 1 c.c. in the case of tyrosine or 3 c.c. in the case of phenol or resorcinol, was deducted from the water dilution used in the Folin protein precipitation method. Control experiments proved that the addition of N/10 HCl in the amounts used, bringing the final solution to at most 0.012 N with respect to HCl, does not interfere with the determination of uric acid. Whenever possible, 10 c.c. of the filtrate were used for the direct method and 15 or 20 c.c. for the isolation method.

While resorcinol as well as tyrosine gives a distinct blue color with the reagents, the color is not of sufficient depth to account for the difference found between the direct and the indirect or isolation method (Tables III and IV) unless we assume a great increase in the concentration of these substances ordinarily present in very small concentrations only. The blue color

TABLE IV

EFFECT OF THE ADDITION OF PHENOL, RESORCINOL OR TYROSINE ON THE COLORIMETRIC DETERMINATION OF URIC ACID IN WHOLE BLOOD

Addition to Whole Blood		FOLIN'S REAGENT					BENEDICT'S REAGENT				
		Determined Uric Acid mg. %		Increase in Uric Acid Determined by Direct Method, Expressed in mg. % Uric Acid per mg. % of Addition			Determined Uric Acid mg. %		Increase in Uric Acid Determined by Direct Method, Expressed in mg. % Uric Acid per mg. % of Addition		
Substance	mg. %	Direct	Isolated	Phenol	Resorcinol	Tyrosine	Direct	Isolated	Phenol	Resorcinol	Tyrosine
No Addition		4.25	2.45	—	—	—	5.24	2.50	—	—	—
Phenol	3.6	5.00	2.50	0.21	—	—	6.75	2.50	0.31	—	—
Resorcinol	2.9	5.32	2.60	—	0.17	—	5.70	2.65	—	0.16	—
Tyrosine	1.0	4.25	2.50	—	—	0.00	5.55	2.50	—	—	0.31
No Addition		3.34	2.00	—	—	—	4.00	2.20	—	—	—
Phenol	2.25	3.75	2.10	0.18	—	—	4.70	—	0.31	—	—
Resorcinol	2.32	3.70	2.00	—	0.15	—	4.55	2.20	—	0.23	—
Tyrosine	1.00	3.34	2.00	—	—	0.00	4.35	2.10	—	—	0.35
No Addition		3.30	1.80	—	—	—	3.75	2.00	—	—	—
Phenol	3.00	4.00	—	0.23	—	—	4.65	—	0.30	—	—
Resorcinol	2.32	3.85	—	—	0.24	—	4.20	—	—	0.19	—
Tyrosine	1.0	3.35	—	—	—	0.05	4.05	—	—	—	0.30
No Additions		4.68	2.73	—	—	—	6.45	2.80	—	—	—
Phenol	3.6	5.40	2.80	0.22	—	—	6.75	2.80	0.36	—	—
Resorcinol	2.90	5.10	2.85	—	0.17	—	—	—	—	—	—
No Additions		2.92	—	—	—	—	4.55	—	—	—	—
Resorcinol	2.90	3.50	—	—	0.20	—	5.10	—	—	0.19	
Phenol	3.6	3.80	—	0.24	—	—	5.75	—	0.33	—	—
Averages				0.21	0.19				0.32	0.19	0.32

given by any of the three substances employed varies in tint from the clear blue color due to the presence of uric acid, and develops much more gradually. It is fully developed within an hour. The color given by phenol is the deepest. One mg. of phenol will give a color equivalent to 0.24 mg. uric acid with the Folin reagent and with the Benedict reagent will give a color equivalent to 0.37 mg. uric acid. The concentration of phenols in the blood varies between 2.5 to 5 mg. per cent. If present in the same concentration as uric acid, it will increase the uric acid value determined by the direct method by 24 to 37 per cent, depending upon the reagent used. If the readings are made within twenty minutes after the reagent has been added, the increase will not be quite as marked. Because of the grayish tinge imparted to the blue color by any of these substances, a difference in shade exists between the standard solution and the blood filtrate solution which renders the colorimetric reading more difficult. The color values of these substances expressed in mg. of uric acid per mg. of substance are shown in Table V.

TABLE V

	FOLIN REAGENT	BENEDICT REAGENT
1 mg. tyrosine	0.10 mg.	0.35 mg.
1 " resorcinol	0.16 "	0.25 "
1 " phenol	0.24 "	0.37 "

These values are the average values of six determinations made on aqueous solutions of these substances and are not based on the increases obtained by the direct method when these substances were added to blood or plasma.

CONCLUSIONS

Inspection of Tables III, IV and V leads to the following conclusions:

1. Substances are present in the protein-free blood or plasma filtrate which are not precipitated out by silver lactate or by basic zinc carbonate. These substances give a blue color with the Folin, as well as the Benedict uric acid reagent.

2. The concentration of these substances varies greatly.

3. They react with different intensity with the two reagents.

4. Phenol, resorcinol, or tyrosine if present will intensify the blue color produced by uric acid with the Benedict reagent. Phenol and resorcinol also react, though to a less extent, with the Folin reagent, but tyrosine gives only a slight color with the Folin reagent.

5. The preliminary isolation of uric acid as a silver salt does not cause an appreciable error in the determination of the uric acid in the blood. The loss that does occur, occurs in the precipitation of the blood or plasma proteins through adsorption on the precipitate. Since the precipitate is far less in case plasma is employed, the loss is also less. The loss occurs during precipitation; this fact is shown by the determination of uric acid of known concentration in a solution of sulpho-tungstic acid of the approximate concentration in the filtrates. This is further substantiated by the analyses carried out on blood and plasma with added uric acid. The percentage of recovered uric acid, determined by the isolation method, agrees within the limits of the experi-

mental error of the method with the percentage recovered as determined by the direct method.

6. No parallelism can be established between pathologic condition and concentration of the unknown substances which also react with the uric acid reagent. The group of pernicious vomiting cases is the only one showing a consistency in the divergence between the two methods.

SUMMARY

The direct method of uric acid determination on blood or plasma filtrate, using either Benedict's or Folin's reagent, invariably gives too high a result. It is shown that either of these two reagents gives a blue color with resorcinol and with phenol. The Benedict reagent gives a color with tyrosine as well. Neither of these three substances is precipitated by silver lactate, and there is no increase in the result of the uric acid determination carried out by the preliminary isolation method on bloods or plasmas to which either of the three substances has been added. In the great majority of cases neither phenol, resorcinol, or tyrosine is present in the blood in sufficiently high concentration to account for the total divergence between the two methods. No definite parallelism between pathologic condition and degree of difference has been observed. A modification of the Folin method of uric acid determination, which eliminates the danger of precipitation in the reaction mixture and produces a color which is stable for twenty-four hours, is described; and the method is adapted to the determination of uric acid present in the urine.

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THE SEDIMENTATION REACTION OF ERYTHROCYTES*

Clinical Applications and a Micromethod

By W. H. Morriss, M.D., and E. H. Rubin, M.D., Wallingford, Conn.

IN THE past few years much interest has been displayed by foreign clinicians and investigators in the study of the phenomenon known as the sedimentation reaction or the suspension stability reaction of the erythrocytes. When fresh blood is treated with an anticoagulative substance and allowed to stand in a column, the erythrocytes settle down, leaving clear plasma above. The extent and velocity of this sedimentation have been found to vary widely under certain physiologic and pathologic conditions.

*From the Gaylord Farm Sanatorium, Wallingford, Conn.
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While credit for the discovery of this phenomenon and for its employment as a clinical test is generally given to Fahraeus,[1] who published a monograph on the subject in 1918, it has been pointed out by several authors that the phenomenon was described by Galen as the crusta phlogistica and is mentioned in English and German textbooks of the first half of the Nineteenth Century. Fahraeus was apparently unaware of this fact and also of the excellent papers beginning with that of 1894, published by Prof. Biernacki[2] of Warsaw on the same subject.

Biernacki prevented coagulation by the addition of powdered oxalate and made his determinations by placing the blood in specially constructed cylinders and measuring the degree of fall of the erythrocytes at thirty minutes and one hour. In 1897 he reported a series of sedimentation tests on seventy-five cases, including four of pulmonary tuberculosis, and tabulated his comparative results with defibrinated and oxalated blood. His conclusions were: that the test had clinical value, that the results were not dependent on corpuscular volume, and that the sedimentation velocity was related to the content of fibrinogen in the plasma and intimately related to conditions of disordered oxidation. Biernacki's work was confirmed by that of his pupil Müller[3] and reviewed by Marcano[4] in his study of corpuscular volumes.

Although the work of Biernacki clearly antedates that of Fahraeus, the latter author originated many important studies both on causative factors and on clinical application, and his papers have stimulated interest in the subject while the publications of Biernacki have passed apparently unnoticed, although reference is found to his work in one of Linzenmeier's[5] excellent papers.

CLINICAL APPLICATIONS

Clinically, the reaction has been employed in the study of a diverse group of pathologic conditions. As it was first suggested as a test for pregnancy, it has naturally been used rather extensively in obstetric clinics. It has been found that the reaction remains within normal limits until gestation has reached about the fourth month when it shows a consistent and marked acceleration of sedimentation velocity. As other and more familiar signs of pregnancy are not lacking at this period, its usefulness as a diagnostic method is slight. It has, however, been employed by some authors as an aid in diagnosis between large pelvic tumors such as myomata and ovarian cysts and pregnancy, as benign pelvic tumors do not alter the normal sedimentation reaction.

The test has had very extensive employment in gynecologic practice and has been found useful in distinguishing between pelvic inflammatory conditions and benign growths (Schumacher and Vogel,[6] Pewny[7]). Rumpf[8] considers that the test offers a valuable means to determine when surgical measures should be employed in inflammatory disease of the pelvis, as during the acute stages the velocity is markedly increased and tends to lower values with chronicity.

In surgery it has been suggested and employed with somewhat doubtful success in differentiating between acute appendicitis and acute adnexal dis-

ease. In the former the test is found to remain practically normal for about the first thirty hours and to be always markedly increased in the latter. (Joseph and Marcus,[9] Haller.[10]) W. Löhr,[11] who has used the test extensively, feels that it is of decided value in surgical diagnosis, that it offers a reliable indication of the severity and acuteness of any inflammatory lesion, and that it may be useful in distinguishing benign from malignant tumors. In this last observation he is confirmed by several authors (Gragert,[12] Haller[10]).

In pediatrics the test has found employment. Gyorgy[13] found that infants of over one month showed a somewhat more rapid velocity of sedimentation than adults. In infants of under one month, on the other hand, there was a definite retardation. This retardation has also been noted by Nadolny.[14] Gyorgy found that any fever process in infants was accompanied by an increase in the sedimentation reaction, but that if other causes could be excluded, a marked increase in velocity could be considered diagnostic of syphilis, as its occurrence was paralleled by a positive Wassermann and Sachs-Georgi reaction and diminished with successful antiluetic treatment.

In the field of general medicine the test has not proved very valuable, as most pathologic conditions associated with fever give an increased sedimentation reaction. As a diagnostic help in the differential diagnosis between ulcer and cancer of the stomach, it has been recommended by Löhr,[11] Hoffgard,[15] Kovács.[16] It seems always increased in florid syphilis and is definitely above normal limits in tabes and general paresis (Paulian,[17] Plaut[18]).

It is in the field of tuberculosis, however, that the test has had widest application, and reports of the study of about 17,000 cases of tuberculosis by this method are on record. In general the opinion of the value of the test is that it offers considerable information as to the activity and extent of the lesion present in the lung. The test has been used routinely for the last year and a half at Gaylord Farm Sanatorium, and a preliminary report of the results obtained has been made by one of the present authors (Morriss[19]). Further experience has added to our interest, and our present records of four hundred cases, with examinations repeated at varying time intervals on one hundred thirty cases, have shown such close agreement with other clinical indications that we have come to attach considerable importance to the test in our estimate of the condition of the patient and his need of treatment.

TECHNIC

An estimate of the value of the test has been confused by a lack of standardization in the methods employed and by the difference in terms used in expressing the results. Fahraeus[1] employs test tubes 17 cm. in length with an internal diameter of 9 mm. as containers of the citrated blood and states the results in millimeters (the measure of the fall of the level of erythrocytes at stated times). Linzenmeier[20] uses short tubes of 6.5 cm. length and 5 mm. internal diameter as containers, giving his results in minutes, an expression of the time within which the top of the column of erythrocytes sinks from the filling mark to a second mark placed empirically 18 mm. lower on the tube. Westergren[21] states his results in mm. but uses thin tubes sealed

at one end, of 30 cm. in length and 2.5 mm. internal diameter, and takes his readings at one, two, and twenty-four hours. Several other authors have proposed certain modifications in technic, but the three methods described are the ones usually employed. In all of the methods the blood is obtained from the arm vein and citrated before the readings are made.

The method employed in routine tests made at Gaylord Farm Sanatorium has been a modified Westergren technic, as described in detail in a preceding article (Morriss[19]). The readings are obtained at one, two, and twenty-four hours from ordinary 1 c.c. serologic pipettes graduated from 1 to 100, filled with citrated blood, and represent the percentage of clear plasma lying above the level of erythrocytes. As the pipettes are filled to exactly the 1 c.c. mark, the reading can be made directly from the pipette graduations, thus avoiding the necessity of measuring the results on a mm. scale. The advantages of a reading thus expressed in percentage also has been emphasized by Fischel.[22] In this technic about 2 c.c. of blood is necessary; it is obtained by venous puncture and diluted with one-fifth volume of 3.8 per cent of sodium citrate.

MICROMETHOD

While venous puncture is usually easy, it was obvious that the value of the test was limited considerably by the necessity of its employment both on account of the lack of familiarity on the part of many physicians with this method of obtaining blood, and on account of the patient's objection to its frequent application. There are also a few cases in which successful venous puncture is very difficult owing to small veins embedded in much subcutaneous fat, or on account of the extreme delicacy of the veins, such as are found in children and infants. Early in our work with the test we turned our attention to the feasibility of devising a micromethod that could be used with small portions of blood, such as were obtainable from the capillaries of the finger tip or ear.

The same need for a micromethod has been felt by several investigators, thus Biernacki[23] has described such a method in which he employed thermometer tubing of ½ mm. internal diameter and used powdered oxalate as an anticoagulant. Marcano[4] in 1901 published perhaps the first micromethod in describing an apparatus which he devised for determining relative corpuscular and plasma volumes, mentioning that it could also be used for determining Biernacki's sedimentation reaction. Fahraeus is quoted by Levinson[24] as having used a capillary tube which required only a drop of blood and yielded results roughly comparable with macromethods. Somewhat similar methods are described by Von Brinckman and Wastl.[25] Balachowsky[26] employed hirudin in a micromethod as an anticoagulant and was unfortunately deterred from verifying his results by the difficulty in procuring this substance. Much more recently methods have been described by Linzenmeier[27] and Kaufman.[28] The former employed a long slender pipette 27 cm. in length and of internal diameter of 1 mm. with a small mixing bulb placed in the center. He diluted with one-fifth volume of sodium citrate mixed by inverting the tube three times and made readings direct from the tube, the mixture being

allowed to settle into the lower half. The test required only two drops of blood and is described as reliable by its author. Kaufman's method is somewhat similar in principle.

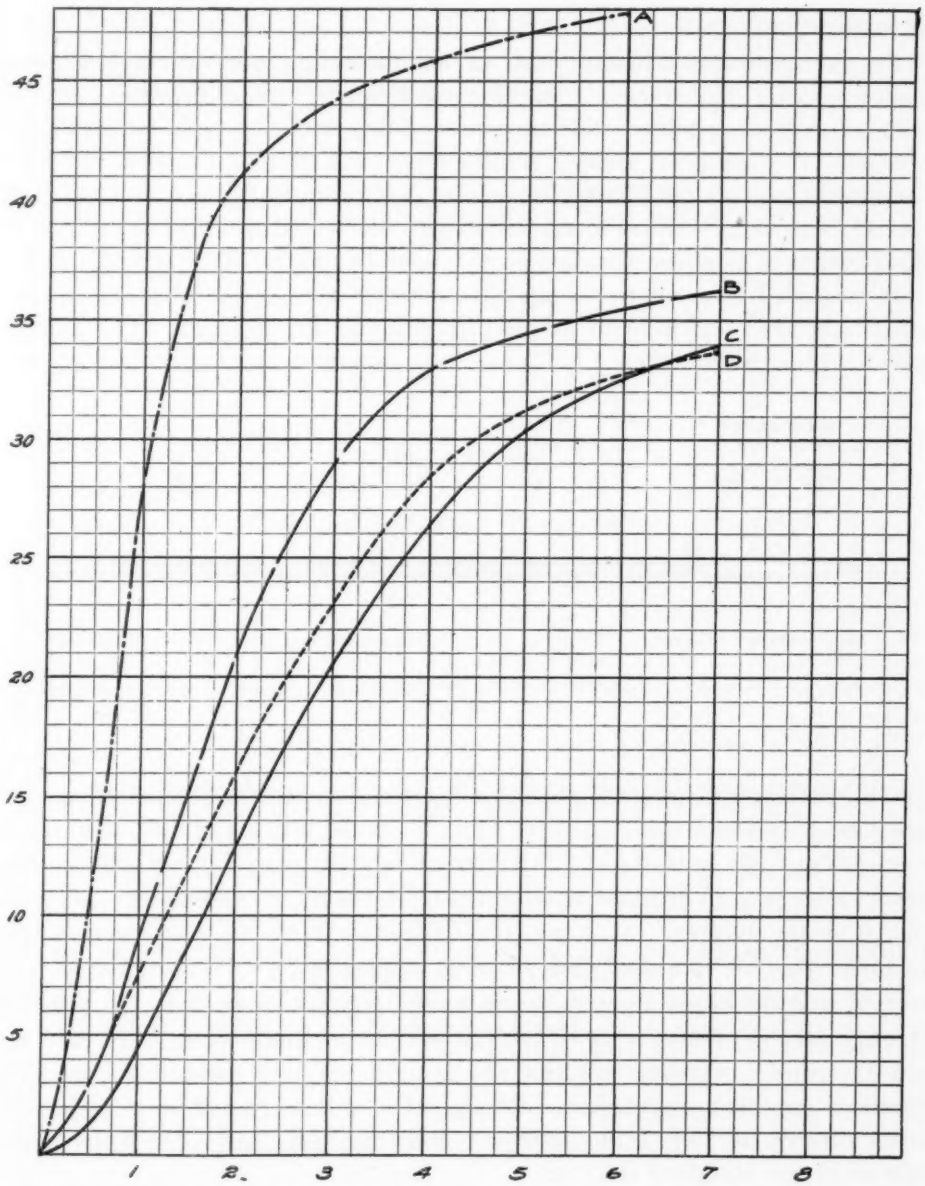

Fig. 1.—A comparison of sedimentation curves in tubes of varying diameter. *A*, Capillary tubing. *B*, 0.1 c.c. pipette. *C*, 1 c.c. pipette. *D*, 0.2 c.c. pipette.
Ordinates represent percentage sedimentation.
Abscissae fifteen-minute time intervals.

In devising such a micromethod two points required definite answer: whether tubes with a very narrow lumen could be employed, and whether blood obtained from capillaries would give results comparable to venous blood. A series of tests was made, using tubes of various diameters such as

0.2 c.c., 0.1 c.c., pipettes and capillary tubing. It was found that the smaller tubes gave a slightly higher percentage reading than the standard method, the differences increasing with the decrease in diameter of the tube; capillary tubes were unsatisfactory, but tubes of a caliber of 0.1 c.c. pipettes gave comparable readings.

Fig. 1 represents a comparison of sedimentation curves derived from tubes of varying diameters: *A*, capillary tubing; *B*, 0.1 c.c. pipette; *C*, 1 c.c. pipette; *D*, 0.2 c.c. pipette. The tubes were filled with the same citrated venous blood. A number of similar results convinced us that the test could be satisfactorily carried out in small tubes employing only 0.1 c.c. of blood. A series of tests using venous blood and finger blood set up in 0.1 c.c. pipettes yielded results in close enough agreement to establish the second point (Table I).

It remained to construct a pipette in which accurate dilution and satisfactory mixture of blood and diluting fluid could be obtained. The pipette devised is illustrated in Fig. 2.* The technic employed in its use is as follows:

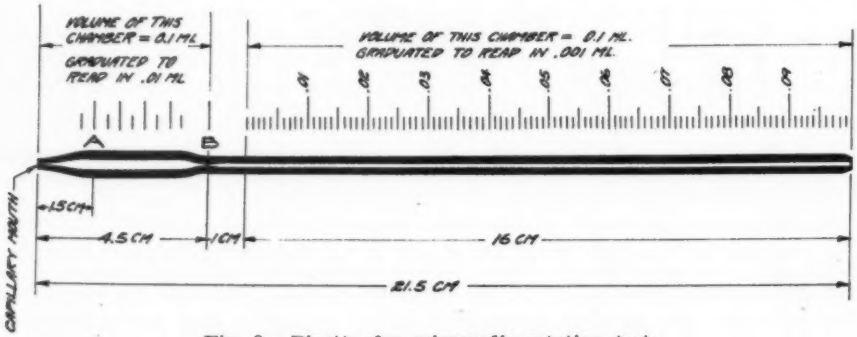

Fig. 2.—Pipette for microsedimentation test.

The patient's fingers are bathed for a few minutes in hot water to produce vasodilatation; the pipette is filled from the bulb end to mark *A*, or to one-fifth of the capacity of the upper calibration with 3.8 per cent sodium citrate; a large drop of blood is obtained from the finger and drawn up to the mark *B* on the diagram. It is important that free bleeding take place so that the blood can be obtained with little or no pressure. The pipette is then inverted and the blood allowed to run down into the lower end. To promote thorough mixing, which is essential, the mixture is then driven back into the bulb by gentle pressure through the mouthpiece and rubber tubing; the process is repeated three times. The pipette is then placed in a rack with spring clamps holding it in position. Readings are made direct from the scale as in the standard technic.

COMPARISON OF MICROMETHOD AND MACROMETHOD

In order to determine the reliability of the micromethod a series of comparative readings were made. Table II represents a comparison of averages in one hundred cases between the micro and the standard methods. The determinations were made by both authors to avoid the personal element in

*Pipette was made up through the courtesy of Arthur H. Thomas Company.

technic. A certain amount of practice in the use of the small pipette was found necessary before satisfactory results could be obtained, but it is a far simpler proceeding than the usual blood count.

It was found that slightly higher percentages were obtained with the micromethod, but that the differences were fairly constant and appeared to depend more on certain physical factors due to the tube caliber than on differences between capillary and venous blood.

TABLE I

COMPARISON VENOUS AND CAPILLARY BLOOD

Figures Represent Percentage Sedimentation as Read from 0.1 c.c. Pipettes

SERIAL NUMBER	1 HOUR		2 HOUR		24 HOUR	
	VENOUS	CAPILLARY	VENOUS	CAPILLARY	VENOUS	CAPILLARY
1	15	11	29	26	50	58
2	18	12	31	27	54	61
3	7	5	18	17	38	55
4	14	8	24	21	47	60
5	17	—	32	37	52	62
6	2	1	5	6	37	51
7	1	—	3	4	28	41
8	30	—	45	55	58	68
9	1	2	2	3	35	43
10	23	—	39	57	57	66
11	7	15	21	29	47	54
12	44	47	58	59	68	68
13	—	14	24	33	55	58
14	—	2	7	3	41	50
15	33	42	50	59	—	—
Average Difference	4.4		5		9.2	

TABLE II

A COMPARISON BETWEEN THE MICROMETHOD AND THE MACROMETHOD IN 103 CASES

READING TIME	NUMBER OF CASES	AVERAGE DIFFERENCE	MAXIMUM DIFFERENCE
1 hour	69	4.9	15.5
2 "	103	7.6	23.5
24 "	71	10.2	25.0

TABLE III

THE RELATIONSHIP BETWEEN SPEED OF SEDIMENTATION AND THE AVERAGE DIFFERENCES IN MICROMETHOD AND THE MACROMETHOD

SEDIMENTATION VALUES %	NUMBER OF CASES	AVERAGE DIFFERENCE
0–10	25	4.5
10–20	32	7.2
20–30	21	9.3
30+	25	9.9

In rapidly sedimenting blood the percentage of difference was increased as shown in Table III. While on the whole the micromethod is not as reliable as the standard macromethod, it is sufficiently so to be employed with confidence for clinical purposes and can be translated into results comparable with the regular technic. It thus offers a method of wider general application than that employing venous puncture and enables us to study the reaction when frequently repeated tests seem necessary.

CONCLUSIONS

1. The sedimentation reaction has found widespread employment as a clinical test and has been found especially useful in tuberculosis as an indication of the degree of activity present.

2. A micromethod is presented employing only 0.1 c.c. of blood which offers results comparable to standard macromethods.

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MICROSCOPIC STUDIES ON CAPILLARY INNERVATION AND STAINING OF THE ENDOTHELIAL CELLS*

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RECENT investigations on the blood capillaries by Krogh, Dale and Laidlaw, Danzer and Hooker, Richards, Boas, Carrier, and others are calling much attention to this field and are serving as a great source of inspiration for further work along clinical lines. No doubt some of the researches have been quite overenthusiastic, especially the work done by the Germans in their studies on the human capillaries as pointed out by Boas. However, something of value may be gleaned from further observations.

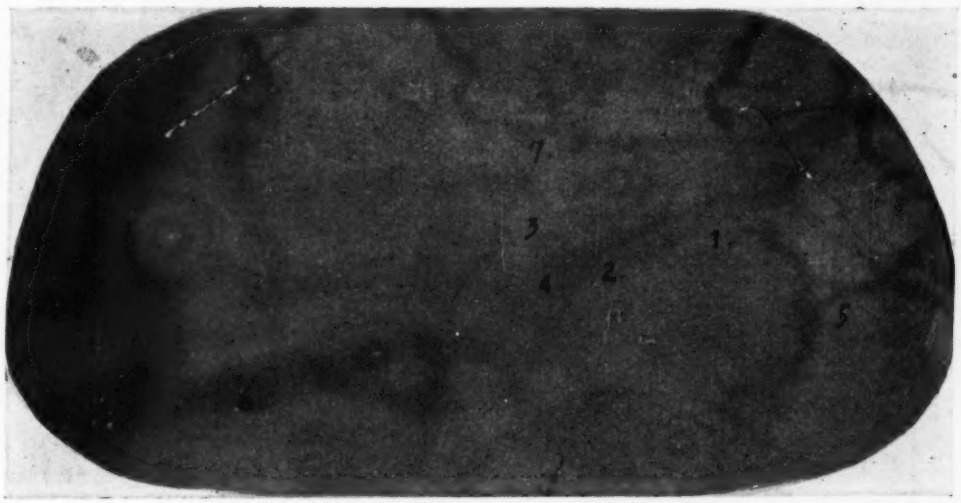

Fig. 1.—Photomicrograph of the frog's tongue (x 160). *1.* Nerve fibril. *2.* Contact of blood capillary with nerve fibril. *3, 4,* and *7.* Network of capillaries in contact with branches from the nerve fibril (*1*). *5.* Arteriole. *6.* Venule. *8.* Vein.

INNERVATION OF THE CAPILLARIES AND ARTERIOLES IN THE FROG

Krogh has demonstrated that cocainization abolishes the responses of capillaries to mechanical, as well as to certain chemical stimuli and has suggested a nervous mechanism due in all probability to axone reflexes. Bruce showed that oil of mustard applied to the skin causes a local cutaneous inflammation which persists after sectioning the dorsal roots of the spinal nerves; this fact suggests that the reaction must have something of an axone reflex nature and consequently could not be attributed to a spinal reflex. After the sensory fibers had degenerated, this effect could not be elicited.

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"A similar axone reflex has been found to occur in certain connector fibers of the thoracico-lumbar outflow which supply the smooth muscle of the bladder, the blood vessels of the rectum, and the internal anal sphincter. These fibers send collaterals to the bladder through the hypogastric nerves. When one of these is cut and stimulated centrally, the muscles innervated by the collaterals in the other hypogastric nerves are seen to respond. There is no evidence, however, that this mechanism is brought into play in normal life." Macleod.

Microscopically, it has been possible to study the distribution of nerve fibers in the frog's tongue and mesentery. With a magnification of 160 diameters larger nerve trunks were located and traced as far as possible to the point of branching. The branches were seen to approach one or more capillaries and touch, and beyond the points of contact they could be traced no

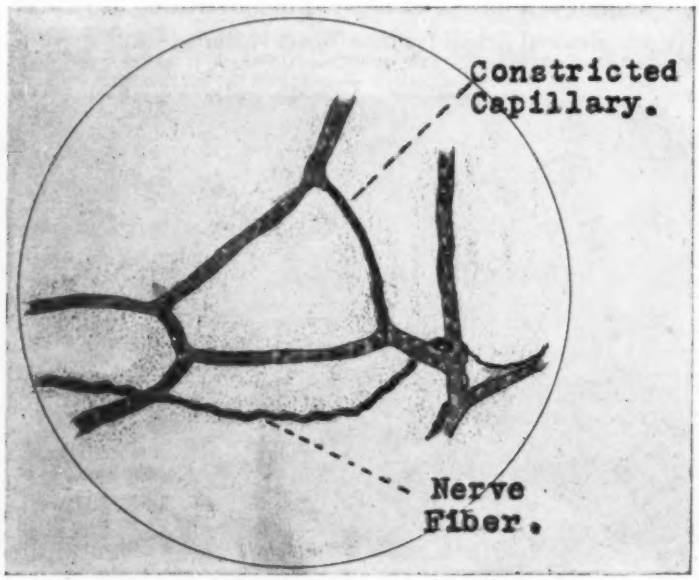

Fig. 2.—Innervation of the capillaries. From the frog's tongue (16 mm. objective, 10x eyepiece).

further. (See Figs. 1, 2, and 3.) Many arterioles, on the other hand, were seen to receive directly into the substance of their walls small collateral-like branches from the larger nerve trunks. (See Fig. 4.)

From these findings we can agree with Krogh that the capillaries are innervated, not necessarily by continuity but by contiguity. The nerve impulse enters the vessel at the point of contact with the nerve fibril and is propagated from there to other parts of the capillary and to other capillaries, the conducting medium being the walls of the vessels. The liability of confusing lymphatics with nerve fibers in the study of the frog's mesentery was borne in mind, and care was taken to differentiate between the two structures. Practically every individual axone process in the nerve fibers was discernible: comparatively few collateral branches are given off, there is a slight tendency towards translucency, and these are seen to pursue a more or

less tortuous course. The lymphatics, on the other hand, differ quite markedly from this description. There is less branching; they are transparent with sharply defined walls, are quite readily stretched, and in the spread mesentery follow a straight course.

STAINING OF THE CAPILLARY ENDOTHELIAL CELLS

Krogh has contributed much in the study of the capillary endothelial cells by outlining the internal surfaces following the injection of india ink. Guinea pigs received injections of the dye intravenously, were then killed and both the fresh and stained tissues examined microscopically, The capillary tubes were simply elongated dark fields, and both endothelial cells and blood corpuscles appeared in striking contrast to the surrounding black medium. Such a method might enable the investigator to make fairly accurate observations on the turgidity of the capillary wall during constriction and dilatation.

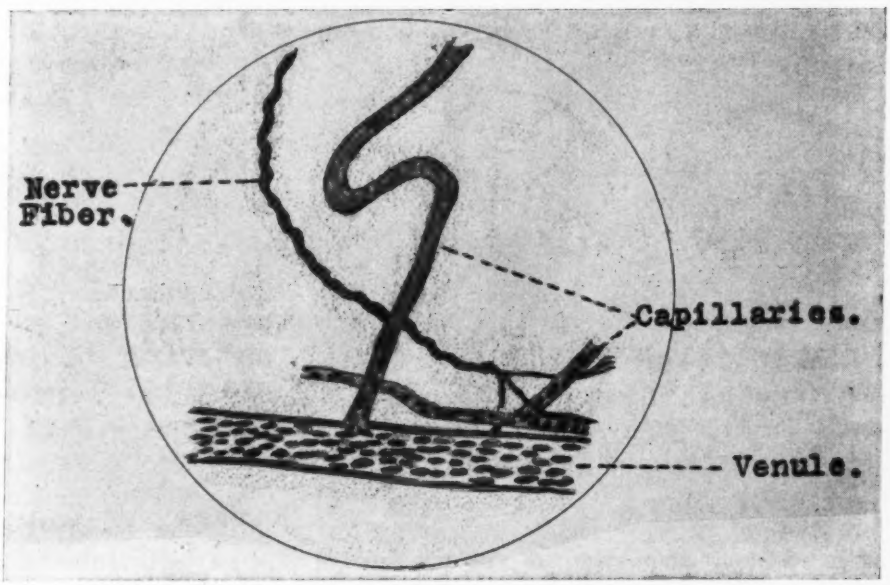

Fig. 3.—Innervation of the capillaries. From the mesentery of the frog (16 mm. objective, 6x eyepiece).

Leonard Hill placed a drop of 20 per cent urethane (ethyl carbamate) on a capillary field which dilated the capillaries and followed this up with an intravenous injection of neutral red. Shortly afterward the bordering tissues of the capillary were stained, with the stain fading away from the same capillary. Krogh thinks that this may be due to increased permeability of the wall. This method enables us to study the physiology of the endothelial cells lining the capillaries in relation to the possibility of their becoming thickened or thinned in capillary constriction and dilatation, in that the ill-defined external surfaces of the cells may be compared with the stained outlying tissues. Certain discrepancies, which are obvious, would arise in the attempt to study these during physiologic activity.

After numerous attempts at staining the endothelial cells in the capil-

laries of the frog, it has been found possible to stain them slightly intra vitam. A number of anilin dyes were tried, but these proved to be too toxic. However, a subcutaneous injection of 0.5 c.c. of a 1 per cent solution of indigocarmin will cause a marked bluing of the thighs and legs as well as a lesser amount of coloration of other parts of the body. At the same time the capillary walls are stained a pale bluish-gray. A number of capillary fields were studied in the web of the frog's foot: magnifications of 100 diameters revealed but little detail; magnifications of 440 diameters brought out the clearly outlined, pale bluish-gray endothelial cells. The most satisfactory method of study, however, was with a magnification of 950 diameters (oil immersion). The surrounding tissues were stained more deeply than the cells under observation. The cells were quite regular in outline except for some

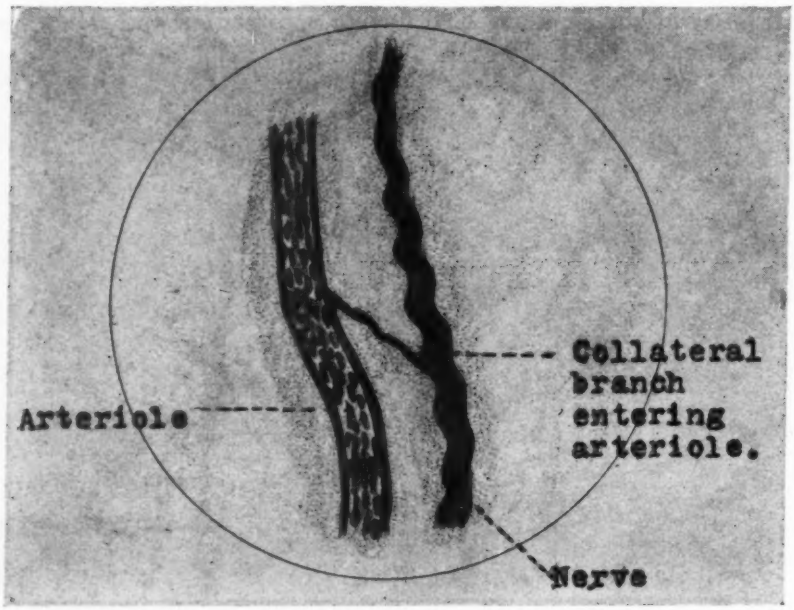

Fig. 4.—Innervation of arteriole. From the frog's tongue (16 mm. objective, 10x eyepiece).

bulging into the lumen of the capillary at short and irregular intervals. The external surface of the cell also presented occasional irregularities. Neither nuclei nor contiguous surfaces were discernible. Another satisfactory method of introducing the dye into the frog is by intracardial injection. No ill effects come from the slow introduction of 0.3 c.c. of a 1 per cent solution of indigocarmin directly through the ventricular wall with an ordinary hypodermic needle. The heart rate is accelerated and the capillaries are slightly dilated after the administration of the dye. With this method of study, no thickening of the endothelium lining the capillaries was observed when they were made to constrict. This disproves the cell-turgor theory which has been advanced to explain capillary constriction and dilatation. As will be recalled, the theory states that in active constriction of the blood capillary, the endothelial cells lining the lumen are capable of imbibing water either from the

blood stream or from the outlying tissues, at the same time becoming so thickened as to encroach inwardly, thereby lessening the diameter of the lumen. Dilatation was explained by the loss of water from the endothelial cell, which in turn caused a thinning out of the cell with the concomitant increase in the diameter of the capillary lumen.

SUMMARY AND CONCLUSIONS

The blood capillaries in the frog's tongue are innervated by contiguity with the nerve fibrils rather than by continuity with them.

The arterioles, on the other hand are innervated not by contiguity, but by continuity with the nerve fibrils.

Five-tenths cubic centimeter of a 1 per cent solution of indigocarmin injected subcutaneously stains the capillary endothelium a bluish-gray color. The same effect may be obtained with an intracardial injection of 0.3 c.c. of the same solution.

Examination of the stained capillary endothelium with the oil immersion lens reveals neither a thickening of the cells during capillary constriction nor a thinning out during dilatation.

MERCUROCHROME IN TREATMENT OF TYPHOID FEVER: A REPORT OF THREE CASES WITH COMPLICATIONS*

By John H. Foster, M.D., and H. A. Chao, M.D., Changsha, China

SOME remarkable results have been reported following the intravenous administration of mercurochrome-220 soluble in various types of infections and infectious diseases, apparently demonstrating that a valuable remedy has been added to therapeutics.[1]

From its demonstrated antiseptic action in the blood, in the bile, and in the intestinal tract we were led to hope that mercurochome would be of value in the treatment of typhoid fever. Furthermore the reaction after the intravenous injection of mercurochrome rather closely resembles the response to foreign protein, which is beneficial in a certain percentage of typhoid fever cases.[2] Typhoid and paratyphoid fevers are quite prevalent in Changsha during the summer and fall months, and the mortality has varied from 10 to 17 per cent in different years. Some more active and effective form of therapeutics than the present dietetic treatment would be warmly welcomed.

We selected two cases of typhoid fever of moderate severity and free from complications and gave to each 5 mg. of mercurochrome-220 soluble per kilogram of body weight in a freshly prepared 1 per cent solution, with the following results:

Case 1.—T. M. L., a student, aged twenty-four years, was admitted on October 24, 1924, on the sixth day of his illness, with symptoms and signs of typhoid. Blood culture

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and Widal were positive to typhosus. A parotitis developed which subsided in about two weeks. He was given two intramuscular injections of milk with good reactions but with no effect on the temperature curve. On November 6, the thirty-eighth day of his disease, he was given an intravenous injection of 20 c.c. of 1 per cent mercurochrome-220, at 2:30 P.M. Two hours later he had a chill and complained of vertigo. The chill ceased at 6:30 P.M., but he had malaise and abdominal distress during the night. About noon the next day he had a loose bowel movement, colored by the dye, but consisting chiefly of blood and mucus, similar to a severe dysentery stool. There was no further hemorrhage, but the abdominal pain continued until late that afternoon. The temperature seemed to be lowered by the treatment (Chart 1), but did not remain normal until more than a week later. He left the hospital well after another week.

CASE 2.—T. M. M., a laborer, twenty-four years old, was admitted on November 1, 1924, with fever and headache of five days' duration. Symptoms and signs were typical of

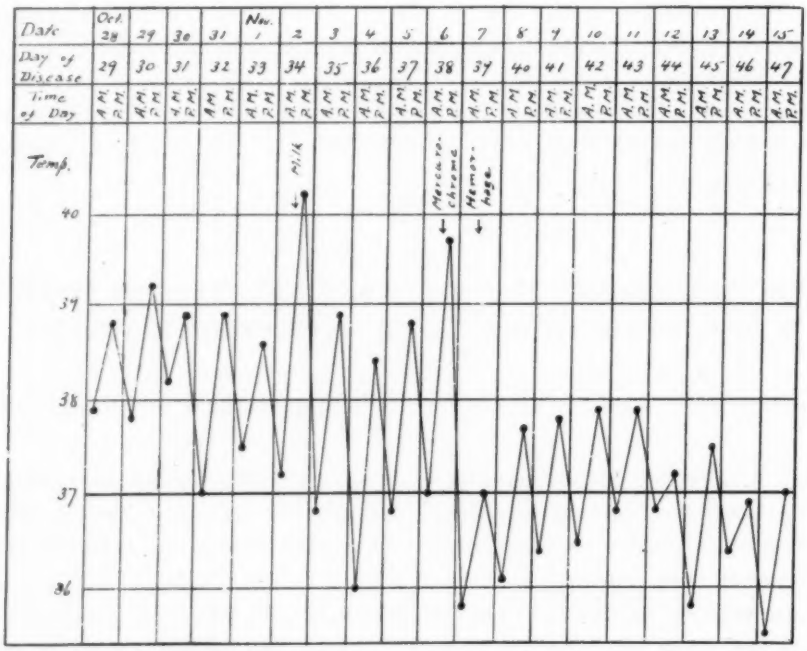

Chart 1.—Temperature chart of T.M.L. (Case 1). Intestinal hemorrhage twenty-two hours after administration of mercurochrome-220 soluble.

typhoid. The blood examination showed 8,200 leucocytes with 61 per cent polymorphonuclears and 39 per cent lymphocytes. Urine and feces were negative. Widal was positive, macroscopically, to typhoid on November 3. There was some abdominal pain at time of admission and he had some retention of urine for which he was catheterized on November 2 and 4. On November 6 at 3:40 P.M. he was given 21 c.c. of a 1 per cent mercurochrome-220 solution, equivalent to 5 mg. per kilogram of body weight. Thirty-five minutes later a chill began which lasted for over two hours. The temperature rose to 39.2° C. His leucocyte count during the chill dropped to 7,400. He complained of malaise and abdominal discomfort. At 9:00 A.M. the next day the temperature had dropped to 36.8° C., and the white cell count had risen to 17,400. At noon, about twenty hours after the injection, he complained of abdominal pain and had a rather profuse intestinal hemorrhage, about 400 c.c. or 500 c.c. of dark blood, with very little fecal matter or mucus. The pain became more intense and rigidity and tenderness appeared. The leucocyte count at 8:00 P.M. was 19,800 with 78 per cent polymorphonuclears. Perforation of the intestine was diagnosed and laparotomy performed about midnight. A perforation was found in the lower ileum. The patient's con-

dition remained critical for several days and he had two intestinal hemorrhages during the week after the operation. He eventually made a good recovery and left the hospital five weeks later (December 22).

With hesitancy we tried intravenous mercurochrome again, not entirely convinced that the two mishaps had been more than an accidental coincidence.

CASE 3.—C. F. C., a coolie, thirty-eight years of age, was admitted on November 9, 1924, with symptoms of eight days' duration. He had a palpable spleen. Rose spots were present, and he had signs of moderate bronchitis. His leucocyte count was 5,800, with 54 per cent polymorphonuclears, 43 per cent lymphocytes and 3 per cent mononuclears. The Widal was first negative but later positive to paratyphoid-A. Mercurochrome-220, 5 mg. per kilogram of body weight, in a 1 per cent solution, was given at 2:30 P.M. on November 20. This was followed by a chill, as in the previous case, in about thirty minutes, but this time there was

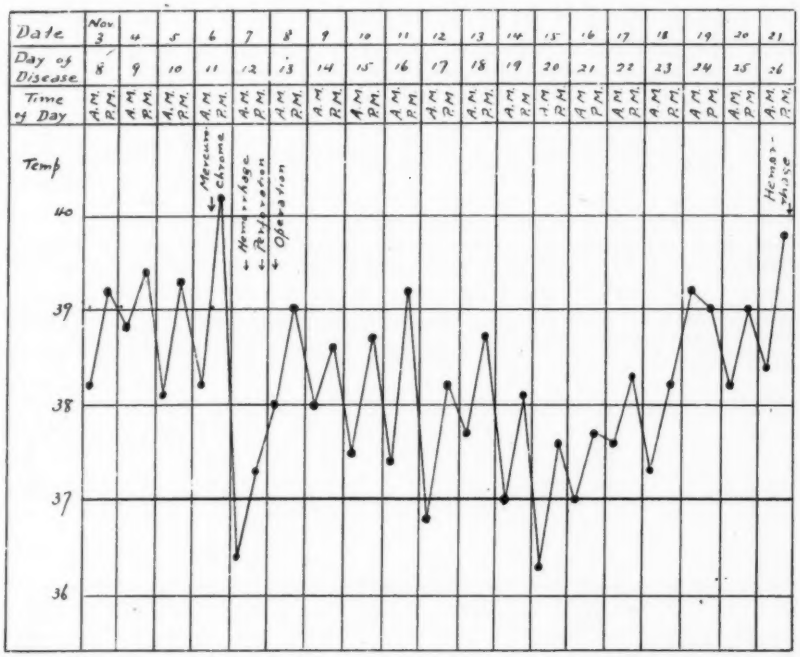

Chart 2.—Temperature chart of a typhoid fever patient (Case 2), in whom intestinal hemorrhage and intestinal perforation followed intravenous injection of mercurochrome -220 soluble.

nausea and vomiting. During the chill the white cell count rose to 13,800. That night the patient had two or three loose watery stools, which were colored by the dye, but which contained no blood. About eighteen hours after the injection he complained of abdominal distress. At noon he had a stool which was composed mostly of blood and mucus and at 8:00 P.M. he had a rather profuse hemorrhage. During the next four weeks a mild diarrhea, with one to four loose stools a day, persisted. Small amounts of blood were frequently present in the bowel movements. The patient left the hospital on December 15. We heard later that he died a few days afterwards following quite profuse intestinal hemorrhages.

The temperature charts of the three cases are presented to show the negligible effect of the injections on the temperature curves.

Leucocyte counts made before and after the injections of mercurochrome-220 are shown in Chart 4. It is interesting to note that in Case 2, the one in whom the perforation occurred, there was a leucopenia instead of the usual leucocytosis during the chill.

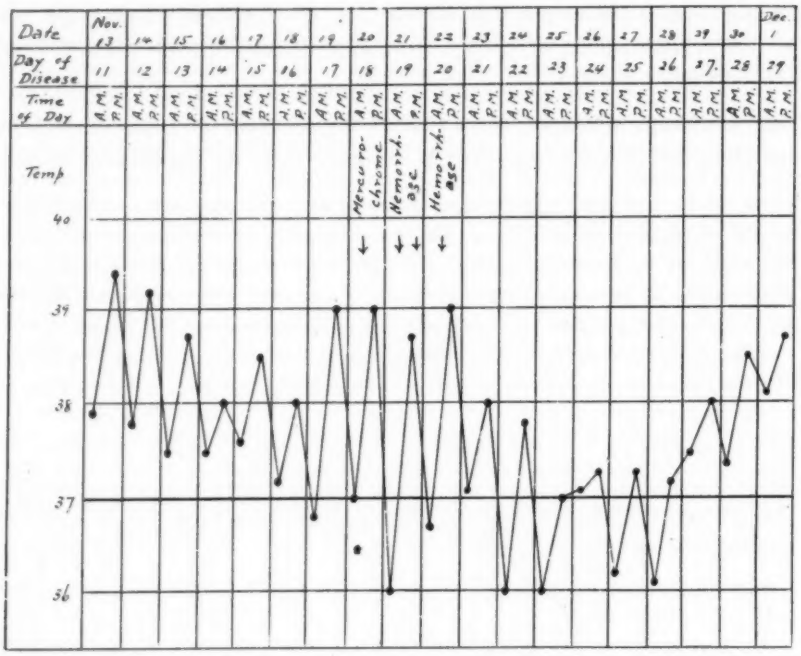

Chart 3.—Showing the temperature curve in the paratyphoid fever patient (Case 3, which terminated fatally), in whom hemorrhages followed mercurochrome treatment.

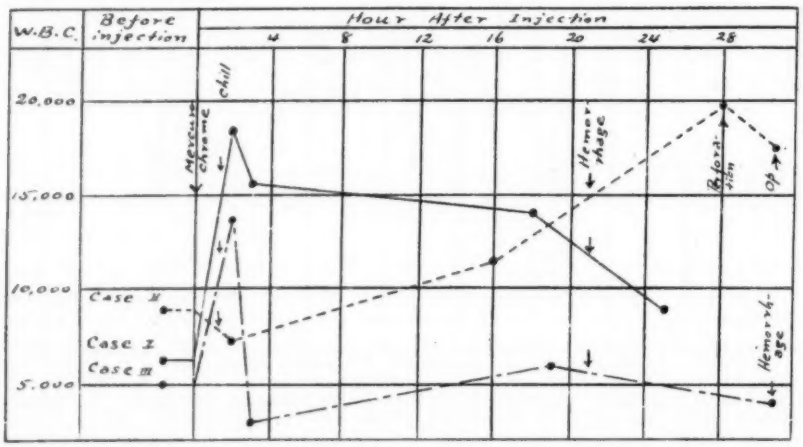

Chart 4.—Showing the leucocyte counts before and after the injection of mercurochrome-200 soluble in two cases of typhoid and one of paratyphoid fever.

SUMMARY

Intravenous injections of mercurochrome-220 soluble, in doses of 5 mg. per kilogram of body weight, were given to two cases of typhoid fever and one of paratyphoid, on the thirty-eighth, eleventh and the eighteenth day of the disease. In none of the cases was there any marked effect on the temperature curve. Two cases showed a temporary leucocytosis during the chill. In all three cases, following the injection, there was a chill with general malaise and abdominal distress. One had nausea and vomiting. About twenty hours after the treatment all three had an intestinal hemorrhage, moderate in one

but quite severe in the others. The first case had no further complications and was discharged in two weeks. The second case had a perforation of the intestine but recovered after operation. In the third there was continued diarrhea and the patient died about four weeks later with more hemorrhage.

COMMENT

These results are quite different from those so far reported for typhoid fever. In the twelve cases treated by Bond and Barrier[3] and in the seven additional cases reported by Young[1] no serious complications were noted.

Three cases are too few to be taken as the basis of any general conclusions, but the symptoms, occurring in three consecutive cases, at about the same interval after the injection and in such varying periods in the course of the disease, seem to be due to the drug. Possibly smaller doses would not have produced such symptoms. Young,[1] however, declares that severity of the diarrhea is due to individual variations in the patients and is not dependent upon the size of the dose. It seems to us that intravenous injections of mercurochrome-220 are contraindicated in typhoid fever and other cases with intestinal ulceration.

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THE STHENIC ACTION OF ADRENALIN ON THE INTESTINES*

By W. J. R. Heinekamp, Chicago, Illinois

THE most generally accepted action of adrenalin on the gastrointestinal tract is inhibition of peristalsis and relaxation of tone,[1-6] the sphincters being contracted.[7] Elliott,[8] using rabbits, and the balloon method for recording contractions, found that adrenalin relaxed the stomach to extreme flaccidity, while the pyloric sphincter contracted. Bech,[9] working with rings from the middle of the frog's stomach, found that strong solutions of adrenalin produced relaxation and decreased tonus. Exceptionally at the beginning, a contraction was effected. Hoskins and McClure,[10] using the balloon method, found that the small intestine was brought to a standstill and extreme dilatation was produced by quantities of adrenalin inadequate to cause a minimal sustained rise in blood pressure. O'Connor,[11] employing excised tissue, rabbits and doves, found that adrenalin effected inhibition of the intestines. Hirtz,[12] using strips of rabbit's pylorus, observed a relaxation with 1:200,000 adrenalin. Trendelenburg and Fleischhauer[13] found the gut to be inhibited even when no change in blood pressure was noted. Schuller,[14] using frog's rectum, noticed inhibition only with adrenalin. Tezner and Turold[15] state that adrenalin in concentration of 1:1,000,000 inhibited the pylorus, but, after acetylcholine, adrenalin effected contractions following an inhibition of short duration. Stross[16] found adrenalin to inhibit the rhythmic movements of frog's stomach (rings) as well as the *pendlebewegungen* and rhythmic fluctuations of dove intestine. Holm[17] never observed anything but inhibition of the human appendix following the use of adrenalin. Carlson[18] stated that adrenalin has a predominantly inhibitory action on the stomach of the cat and dog, and a motor action in the rabbit. He states also that the splanchnics, like the vagi, carry both motor and inhibitory efferents to the stomach.

A group of investigators have, however, found the opposite effect. Bunch[19] asserted that suprarenal extract freshly prepared, when injected into animals, always produced systolic tone of gut. Ott[20] also mentions this finding, but neither of these authors advance an explanation. Magnus[3] mentions a single instance in which increased peristalsis resulted from the use of adrenalin. Hoskins[21] states that epinephrin in remarkably high dilutions inhibits peristaltic activity of isolated segments of rabbit's intestine but that still higher dilutions augment often the rhythmic activity. He explains the augmentation produced as being due to stimulation of the metabolic processes of the muscle itself by quantities of the drug too small to affect the sympathetic myoneural junctions, the efficiency of the tissue being thereby increased. Smith[22] found that dilute solutions of adrenalin produced varying

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changes on various parts of the stomach of different animals. Gunn and Underhill[23] say that adrenalin relaxed the isolated small intestine of rabbits, but that it contracted the muscularis mucosae of cats' intestines. Tashiro[24] found segments of catgut suspended in Ringer's solutions to have increased tonus and sometimes augmented movement of the circular muscle following the use of adrenalin in dilute solutions. He thinks the drug acts on the motor sympathetics. Katsch,[25] using the x-ray, found adrenalin to effect motor inhibition of the gastrointestinal tract.

The augmentor effect of adrenalin is considered by Gruber[26] as due to that drug stimulating in weak solutions the augmentative nerve fibers arising from the thoracolumbar autonomic nervous system.

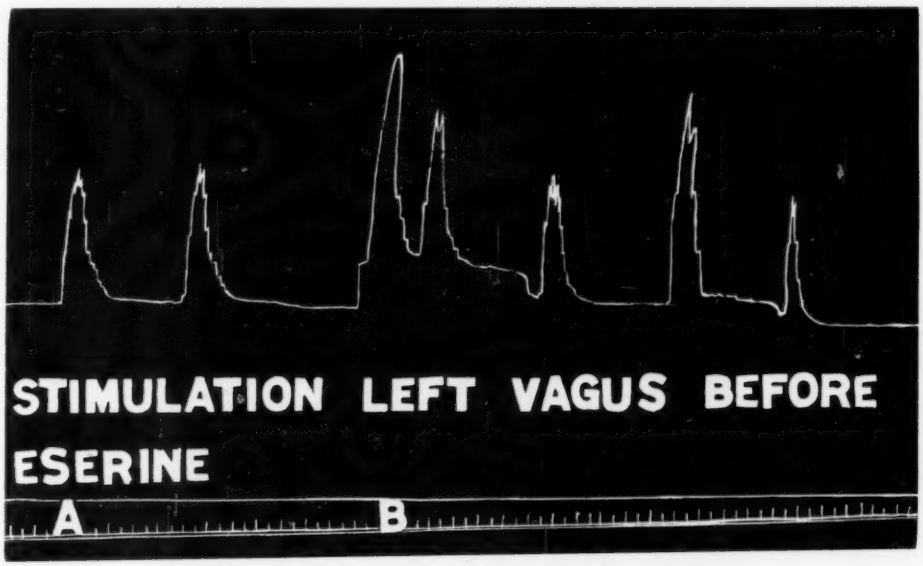

Fig. 1.—Change produced by stimulating intact cervical left vagus. Coil at 12 cm.

In general, the work to date, most of it on excised tissue of rabbit or frog, indicates: (1) that adrenalin ordinarily relaxes the gastrointestinal canal with the exception of the sphincters, which are contracted; and (2) that adrenalin in very minute quantities causes contraction of the gut. This is most generally explained by stating that adrenalin in very dilute solutions effects contractions by stimulating the motor sympathetics, and produces inhibition by stimulating the inhibitory sympathetics, the action in either case being on the myoneural junctions, as suggested by Dixon.[27]

In recent work[28] we found that adrenalin stimulated in a marked degree the peripheral parasympathetic apparatus to the heart. While working on intestinal peristalsis we have occasionally observed an augmentation in tone and an increase in peristalsis, following the intravenous injection of minute doses of adrenalin.

The present investigation was done to determine the cause of this sthenic action of adrenalin.

METHODS

In the experiments, well nourished dogs weighing from 10 to 15 kg., and under light ether anesthesia were used. The recording of respiration and blood pressure was by the usual methods. All drugs were injected intravenously through a cannula inserted into the femoral vein, and were washed in with 5 c.c. of warm physiologic saline. The stomach tracings were recorded by the modification of the Trendelenburg method described by Love.[29] It is

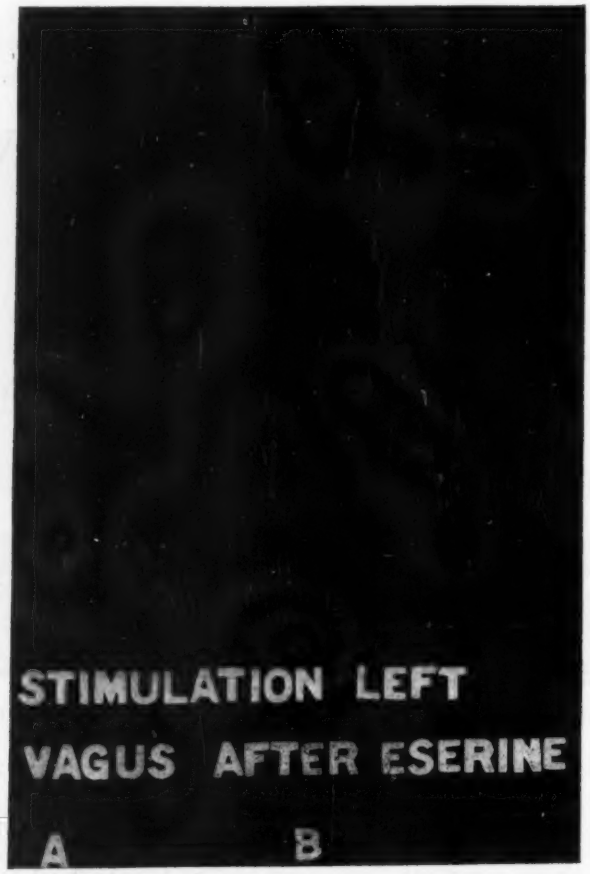

Fig. 2.—Change produced by stimulating intact left vagus after a sensitizing dose of eserine.

our opinion that this method is most simple, since the suturing may be carried on within the abdominal cavity, and the technic is very easily executed. The stomach does not chill and is not subjected to trauma. The animals were fed chopped meat about forty-five minutes before they were used. A portion on the anterior wall of the stomach about 2 inches from the pylorus was used. The stomach was always returned completely to the abdominal cavity and the opening in the belly wall closed as much as possible with sutures. Towels which were kept hot with water were wrapped around the pipe and covered the entire wound. In this manner the stomach did not become cold. The

vagi were usually cut in the cervical region, since we found the contractions to be more marked after this procedure. The splanchnics were left intact.

Electrical stimulation of the peripheral end of the cut vagus was usually carried out with the secondary coil at 12 cm. and the current furnished by a storage battery.

RESULTS

In general, our results were as follows: Large doses, e.g., one c.c. 1:10,000 of adrenalin, always effect a cessation of peristalsis (Fig. 3) and usually a de-

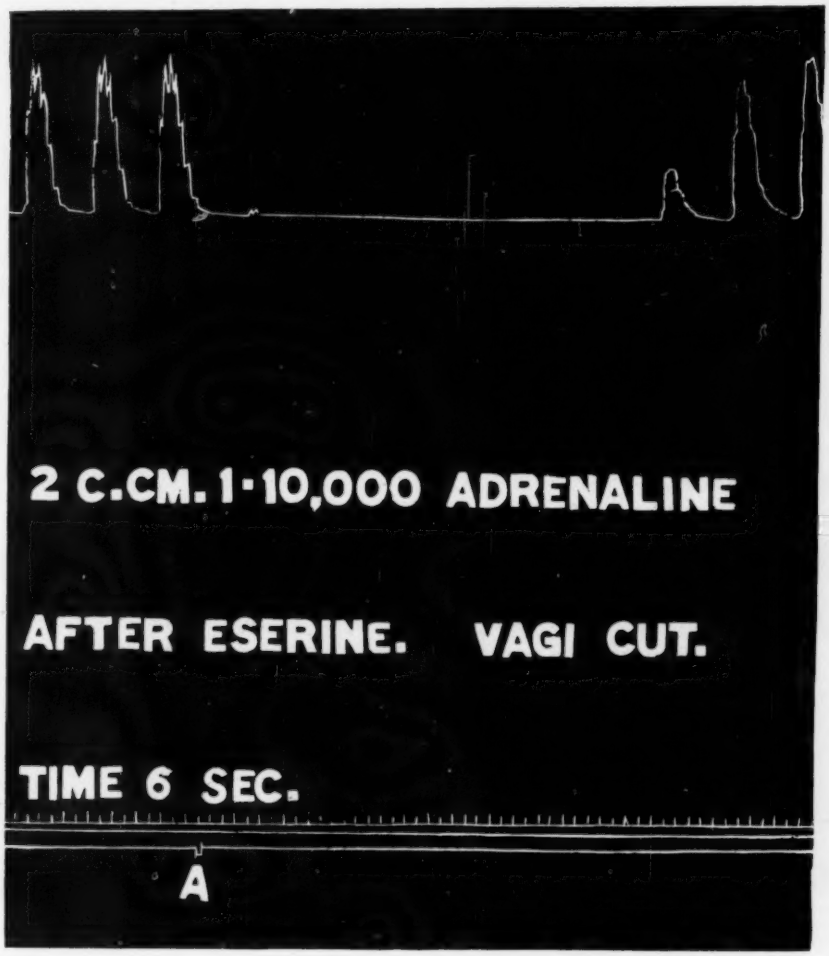

Fig. 3.—Inhibition of peristalsis produced by adrenalin injected at point *A*.

crease in tone. Very minute doses, e.g., one c.c. 1:1,000,000, produce the same effect in many cases. This amount, however, usually produces an increase in tonus and often an increase in peristalsis that is of short duration (Fig. 4). After small doses of eserine, e.g., ½ c.c. 0.1 per cent in a dog of 15 kg., small doses of adrenalin more frequently produce marked increase in tone and peristalsis (Fig. 5). It is usually of short duration, but in one experiment the action was prolonged (Fig. 6), and in another the gut became tonically con-

tracted for a period of three minutes, being relieved by atropine sulphate. In no experiment have I seen this action of adrenalin after the use of atropine.

Stimulation of the vagi after eserine effects a more marked increase in peristalsis and tone, indicating sensitization of the peripheral apparatus by the drug.

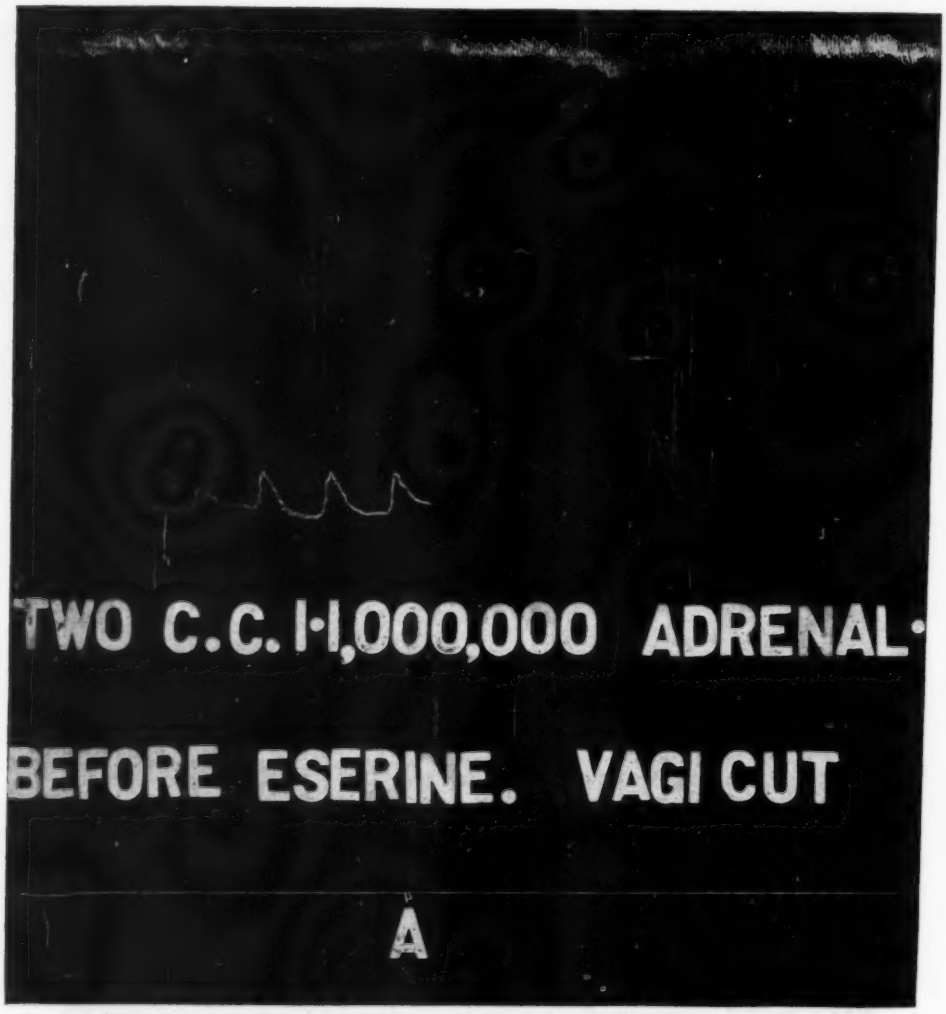

Fig. 4.—Change produced by adrenalin injected at point *A*. Note increased tonus and peristalsis.

DISCUSSION

Stimulation of the vagus nerve effects various results as far as the stomach is concerned. There may be a primary period of decreased tone and peristalsis followed by a secondary period of increased activity, the most common change. There may be no effect during stimulation followed by increased contractions after cessation of the stimuli, or there may be a marked increase in tone and peristalsis immediately on stimulating the vagus. We consider any one of these reactions normal, the latter being the less frequent.

In view of the fact that physostigmine causes contraction of plexus-free preparations, it is conceded that the drug acts upon the nerve endings.[30, 31] In 1915, Langley and Kato[32] found that physostigmine lowered the threshold of nerve excitation. In some previous work[28-33] we found the peripheral

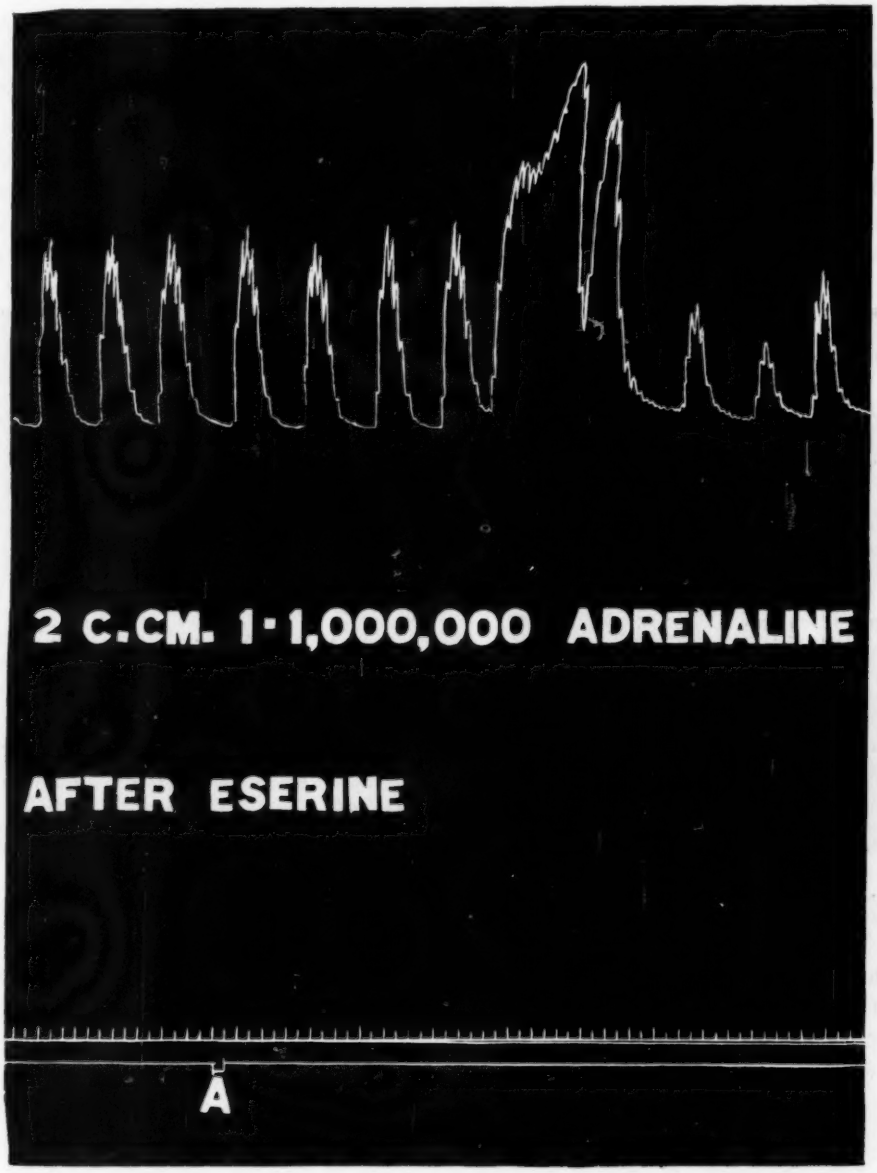

Fig. 5.—Change produced by adrenalin after section of both vagi, and after a sensitizing dose of eserine.

vagus apparatus to the heart sensitized, i.e., the threshold to electrical stimulation was lowered. We believe the vagus to the stomach to be similarly affected, in view of the fact that stimulation of the cervical vagus usually effects a greater reaction from the intestine after a small amount of eserine

has been administered than before. This effect may be seen following the use of 0.15 to 0.20 mg. per kg. of body weight, an amount which in itself produces no effect.

Adrenalin may effect inhibition by: (1) direct action upon the muscle; (2) stimulation of the motor sympathetics; or (3) stimulating the motor parasympathetics. In view of the fact that the sympathetics and parasympathetics are relayed through the metasympathetic system, i.e., the intrinsic nervous apparatus of the intestine, we shall consider the last as part of either of the other two systems.

The usual effect produced by adrenalin in a dose of 0.1 mg. is a marked inhibition of peristalsis and decrease in tone due to stimulation of the myoneural junctions of the sympathetic nerves, as proved by Dixon.[34] With small doses, however, as stated above, adrenalin actually produces an in-

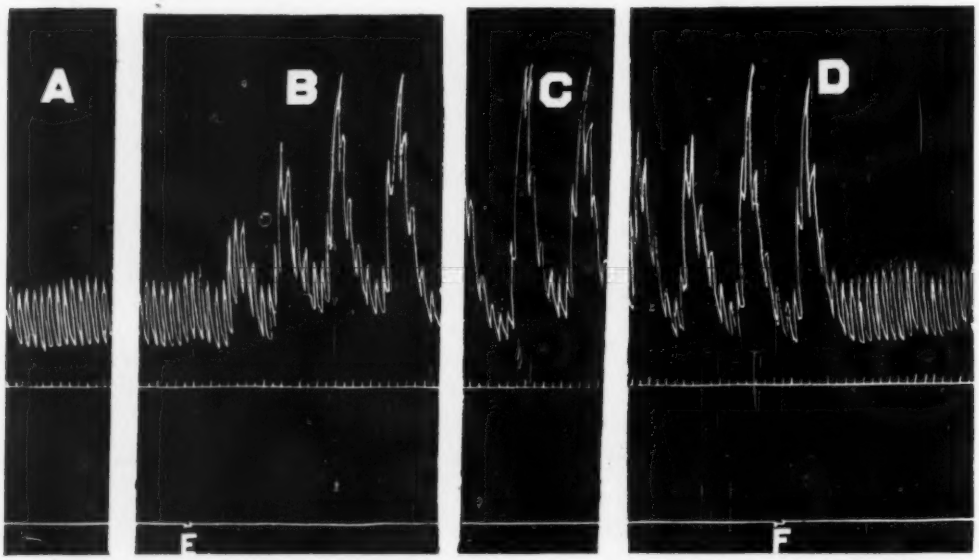

Fig. 6.—Changes effected by adrenalin and atropine. This was the most marked result obtained. *A*, before adrenalin; *B*, immediately after injection of adrenalin at *E*; *C*, three minutes after; *D*, change effected by injection of atropine at *F*.

creased activity of the intestines. This we think is not due to the stimulation of the motor sympathetics nor to direct changes in the muscle, since it is increased after eserine (synergy) and is totally prevented by atropine as shown in Fig. 5.

We do not believe that adrenalin produces inhibition by direct action of the muscle since it does not inhibit the contractions effected by barium chloride (Sollman, p. 331). Nor do we think it due to metabolic changes in the tissue, as advanced by Hoskins, for reasons amply given by Gruber.

In view of the fact that after small doses of eserine, contraction of the stomach is more active than before it, while stimulation of the thoracic sympathetic chain effects the same change, we believe that eserine in these amounts affects the parasympathetics only. And since this action of adrenalin is promptly relieved by amounts of atropine which render the vagus inactive

to electrical stimulation, we believe that the site of action is the peripheral parasympathetic apparatus.

It is our opinion that the effect, whether sympathetic or parasympathetic, depends upon the amount of adrenalin used. We believe that adrenalin stimulates both the parasympathetics and the sympathetics simultaneously, the latter more markedly. The threshold of the motor nerves, however, is lower to adrenalin activity, but its maximal reaction is much less than that of the inhibitor nerves. That is, a dose subminimal for the sympathetics will exert a maximal effect upon the vagus, but a maximal dose for the sympathetics exerts the same maximal parasympathetic change that a smaller dose will.

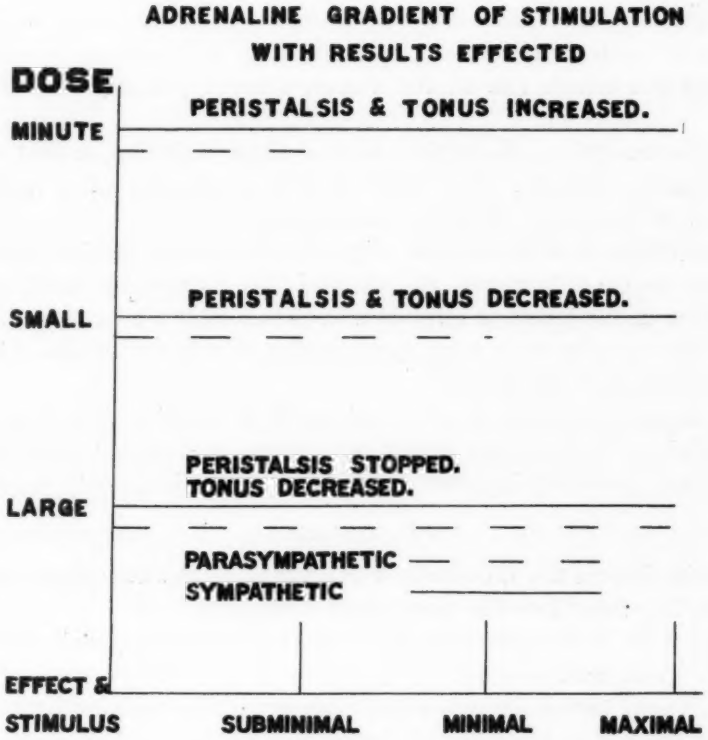

Chart I.

Increasing doses do not effect a proportionally greater reaction from the vagus, as they do from the sympathetics. While it takes a greater amount of adrenalin to act on the inhibitors than on the activators, a more marked reaction is obtained when the action of the latter is obtained. Thus, subminimal sympathetic doses are maximal parasympathetic doses, as are maximal sympathetic doses. The threshold gradient for parasympathetics does not change, while that of the sympathetics does. This is represented graphically in Chart I.

Another factor which must be taken into consideration is the blood pressure. Bayliss and Starling[35, 36, 37] have shown that anemia causes a cessation of intestinal movements. They found that raising the pressure by

compressing the abdominal aorta caused a diminution in intestinal activity. They concluded also that the primary inhibition following stimulation of the vagus was due in part to the decreased blood supply to the gut. In our experiments the amount of adrenalin producing increased activity did not affect the blood pressure sufficiently to consider it as an important factor (Fig. 6). A rise or fall of but a few mm. of Hg. usually resulted, but never any marked change. So we have not considered the blood pressure as a causative factor.

The adrenalin augmentation does not appear immediately after injecting but after a latent period equal to two to three intestinal waves. This also suggests vagus activity, in view of the fact that usually following vagus stimultaion there is a latent period before contractions begin. This normal action of the intestine has been emphasized by Bayliss and Starling. It would seem to indicate also that adrenalin does not influence the inhibitory fibers to the gut which run in the vagus, since a primary decrease is not noted.

With one exception, the action was of short duration, a fact which indicates adrenalin activity. The usual period of augmentation endured for a time equal to the length of an adrenalin curve.

The possibility that this action might be due to the eserine must be considered. In no case, however, do we find the eserine by itself producing changes either in the heart or intestines in the amount we employed. Physostigmine action usually lasts for a period of a few hours, while this persists for a few minutes at the most.

Large doses of eserine or repeated small doses give rise to marked intestinal activity. Under these conditions adrenalin does not further increase the movements, probably because the eserine action is already maximum.

CONCLUSIONS

1. Eserine lowers the threshold of the vagus to the intestines to electrical stimulation, i.e., sensitizes the peripheral apparatus.

2. In minute doses, adrenalin stimulates the peripheral vagus apparatus and is synergistic with eserine.

3. The vagus threshold is normally lower to adrenalin, but the maximal effect is so much less than that of the sympathetic nerves that the action of the latter predominates unless very small amounts of adrenalin are used.

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THE ELECTROCARDIOGRAM IN CORONARY THROMBOSIS*

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WITHIN recent years, coronary thrombosis has become a recognizable clinical condition, yet the diagnosis in many instances is first made at the postmortem examination.

As the clinical history and physical findings of coronary occlusion are frequently insufficient for a positive diagnosis, a method of greater precision is desirable. For this purpose the electrocardiogram has been used. Several different forms of abnormal curves, considered typical of this cardiac disorder, have been published. A variety of irregularities of rhythm and conduction have been observed which, however, are not peculiar to, or diagnostic of, this disease. The occurrence in this disorder of different types of extrasystoles is not uncommon. In four patients reported by Robinson and Herrmann,[1] they observed ventricular tachycardia. The type of curve considered by many as indicating arborization block was frequently observed in this disease by Herrick.[2] The electrocardiographic change considered diagnostic of coronary thrombosis has been confined mainly to alterations in the T-waves.

Clinical Observations.—In an individual with thrombosis of a coronary artery, Pardee[3] observed most striking alterations in the T-waves of the patient's electrocardiogram. The first tracing, obtained on the fourth day of the patient's illness, showed altered T-waves in Leads I and II. These waves were increased in height and branched directly from the R-wave high up on

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its descending limb. The descending limb of the R-wave was much shorter in Lead III than in Lead II. Fortunately Pardee had the opportunity of obtaining tracings during the succeeding sixteen days and observed a progressive decrease in the size of the T-waves and a steady descent of the R-T branching to isoelectric. In the final stage observed, the T-waves were inverted. The inverted T-waves presented steep straight sides and an acute apical angle. The numerous electrocardiograms from this patient show that no single alteration in the tracings is to be expected from patients suffering with acute coronary occlusion.

It is believed by Kahn[4] that the electrocardiogram shows a characteristic alteration in thrombosis of a coronary artery. This he considers as an initial increase in the height of the T-waves which later become sharply inverted and finally return to isoelectric. The curves which he has published show a moderate increase in the height of the T-waves in Lead I with a branching of the T-waves off from the descending limb of the R-waves. In Leads II and III a deep S-wave is present, the ascending limb of which rises above isoelectric and the T-wave branches directly off from the upper end of this elevated limb. This curve resembles that obtained by Pardee at about the eighth day of his observations on his patient.

In Wearn's[5] series of ten cases of coronary thrombosis, there was some alteration in the T-waves of each electrocardiogram. The change in some cases was present in Lead I, in more cases it was present in Leads I and II. In a few of the tracings the T-waves were inverted, while in others they were isoelectric or diphasic. In only one record did he note the high branching of the T-wave directly from the descending limb of the R-wave and then in only the second lead where it disappeared within twenty-four hours. It was Wearn's belief that there was no form of the electrocardiogram characteristic of coronary thrombosis.

In a recent article, Pardee[6] restates his belief in the characteristic alteration of the T-wave of the electrocardiogram by coronary thrombosis. He has found that the change occurs in one or more, but usually only in one lead. He describes what he considers the typical change as a downward, sharply peaked T-wave immediately preceded by an upwardly convex S-T or R-T interval. If this condition is present in Lead III alone, he considers it of no significance, but if the T-wave is also inverted in Lead II, even though this latter is not preceded by this upwardly convex R-T interval, he considers it of diagnostic importance.

The occurrence of ventricular tachycardia with coronary thrombosis was the point dwelt upon by Robinson and Herrmann[1] in their report of four cases. The electrocardiogram of their Case 1 obtained after the cessation of the tachycardia does show a branching of the T-waves directly off from the descending limb of the R-waves in Lead I. This condition is even more pronounced in Leads I and II of the electrocardiogram taken in their Case 2 after the tachycardia had stopped. They state that Cases 1 and 2 showed the most evidence of obstruction in the coronary arteries.

In the nine cases of coronary thrombosis recently reported by Willius,[11] his published curves show branching of the T-waves directly off from the R- or S-waves with absence of the isoelectric R-T or S-T interval in four cases and, as he states, this is such a definite alteration that its presence should always direct attention to the possible existence of acute coronary obstruction. The inversion of the T-waves in one or all leads was present in eight of his nine cases.

In considering the electrocardiograms of coronary thrombosis, it is necessary that caution be followed and that the diagnosis be confirmed as far as possible by our clinical methods or by autopsy. All cases where doubt may arise as to the accuracy of the diagnosis should be excluded from consideration. We present the following cases, the diagnosis having been made either on histories and physical findings generally considered as typical of this

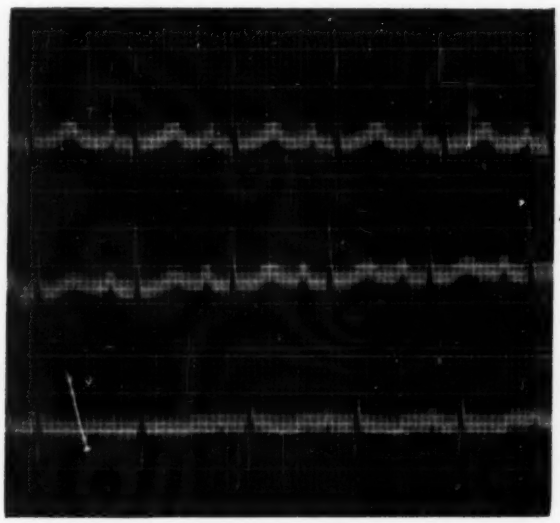

Fig. 1.—Electrocardiogram taken eight months before the attack of severe heart symptoms.

condition, or on autopsy. The electrocardiographic changes in these patients are those which we have come to consider as strongly suggesting this disease. One of our patients may possibly be placed in the second group of Herrick's classification as he died within a few hours after the probable onset; the others belong in his fourth group, the patients having recovered and regained a slight amount of cardiac efficiency.

CASE REPORTS

CASE 1.—An insurance salesman, aged fifty-one. This man was admitted to the hospital complaining of a recent attack of severe agonizing precordial pain which lasted several hours. He also complained of dyspnea and edema.

History.—His only past illness had been typhoid fever. He was examined by his home physician in May, 1923, at which time his heart was found to be enlarged and his exercise tolerance poor. He had no edema but did complain of dyspnea. His blood pressure was 150 systolic and 90 diastolic and his heart rate was 100. He improved by rest in bed. His

present illness started in December, 1923, as a sudden, severe, persistent and agonizing substernal pain, not relieved by nitrites.

Examination.—He was a man of large frame and moderately obese. No dyspnea was observed. The skin had a sallow, pasty color. The chest was barrel-shaped, the lungs being moderately emphysematous but showed no congestion at their bases. The left border of cardiac dullness extended 15 cm. from the midsternal line in the fifth interspace. There was a gallop rhythm, the heart sounds being very distant. The radial arteries showed moderate thickening. The blood pressure was 125 systolic and 100 diastolic and there was edema of both ankles. Dyspnea could be produced on the slightest exertion and the vital capacity of the lungs was 2,200 c.c. No friction rub was heard.

E. K. G.—An electrocardiogram taken by his home physician in May, 1923, is reproduced in Fig. 1 and shows no definite abnormalities. In Fig. 2 is a second curve taken in December, 1923, eighteen days after his attack of severe precordial pain. This tracing shows inversion of the T-waves in all leads.

Course.—This patient continued in a state of severe myocardial insufficiency and died in May, 1924.

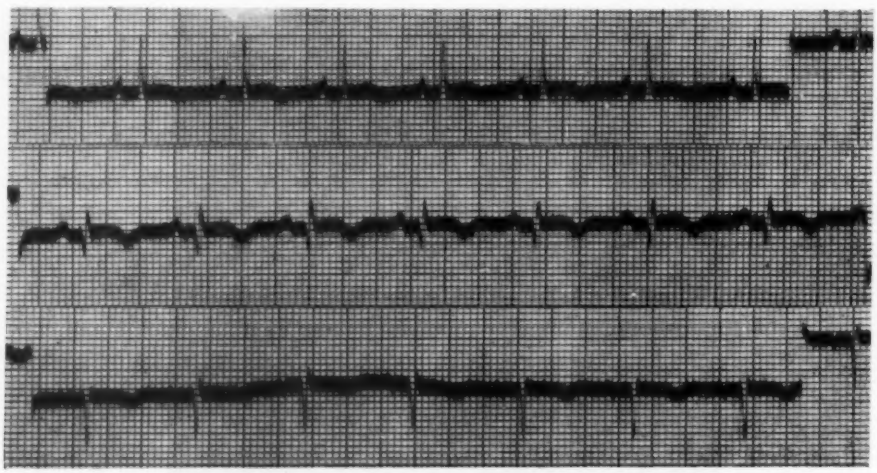

Fig. 2.—Electrocardiogram taken eighteen days after onset of heart symptoms.

CASE 2.—An elderly man, with no occupation, aged sixty-five. He first entered the hospital on July 30, 1924, complaining of an attack of severe agonizing substernal pain which had lasted several hours and was not relieved by any medication. He also was very weak and had dyspnea, edema, gastric distress and flatulence.

History.—He had severe pneumonia at the age of twenty-five, but otherwise had been unusually healthy up to his present illness.

The present trouble had started on July 24, 1924, when suddenly and without provocation he was seized by a severe, cutting, almost unbearable, pain in the substernal region. This pain lasted several hours and was accompanied by dyspnea and orthopnea. He further stated that with the attack, his heart "fluttered" and his lips were purple.

Examination made on August 2, 1924, showed a short, obese man who was suffering from severe dyspnea and orthopnea. His lips and extremities were extremely cyanosed. The apex impulse of the heart was not seen or felt. The left cardiac border was 14 cm. from the midsternal line in the fifth interspace. The heart rhythm was regular, the sounds distant and of very poor quality. There were no murmurs. The blood pressure was 110 systolic and 70 diastolic. The heart rate was 100. The lungs were emphysematous with moisture at both bases. The liver was tender, its edge being felt 2 cm. below the costal margin. The radial arteries were thickened and edema was present in the legs. No elevation of temperature was noted.

E. K. G.—The first tracing (Fig. 3) taken August 5, 1924, showed a branching of the T-waves off from the descending limb of the R-wave in Leads I and II, there being no isoelectric R-T interval. On February 20, 1925, a second curve (Fig. 4) showed a flattened, almost isoelectric T-wave in Lead I, the branching of the T-waves directly off from the R-waves in Leads I and II having disappeared.

Course.—Up to February, 1925, the patient continued to show dyspnea, which increased on slight exertion, the liver remained enlarged and edema persisted in the legs but there had been no recurrence of the chest pain.

CASE 3.—Attorney, aged fifty-nine. He entered the hospital on February 5, 1925, complaining of dyspnea, orthopnea and edema.

History.—He had had rheumatism of unknown type and gonorrhea as a boy. Symptoms of heart disease had first appeared in March, 1923. He had been previously admitted to the hospital in May, 1924, complaining of cough and dyspnea. Treatment afforded him considerable relief. These symptoms recurred with greater severity but a few weeks before his last admission, with also palpitation, nausea and vomiting. There was no complaint of pain.

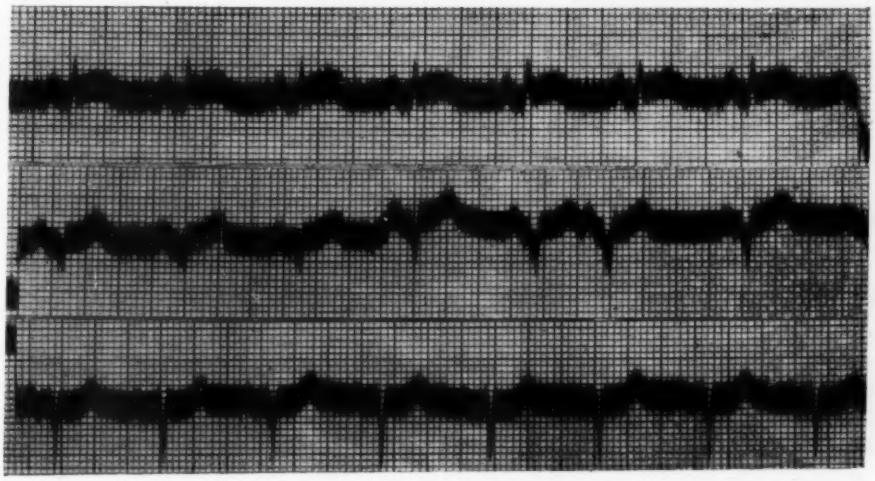

Fig. 3.—Electrocardiogram taken a few days after onset of heart symptoms.

Examination.—He was moderately obese. The pupillary reaction to light and tendon reflexes all gave normal responses. Dyspnea and cyanosis were very evident with increased pulsation in the great vessels of the neck. The apex impulse could not be localized. The left border of cardiac dullness was 15 cm. from the midsternal line in the fifth interspace. The heart rhythm was regular, with frequent extrasystoles, the second aortic sound being accentuated. A blowing systolic murmur was heard at the apex, replacing the first sound. No friction rub was heard. The blood pressure was 150 systolic, 95 diastolic, with palpable thickening of the radial arteries. Edema was present at both lung bases and pitting edema in both lower extremities. The liver edge was felt 4 cm. below the costal margin. The blood Wassermann reaction was negative.

E. K. G.—The one electrocardiagram was obtained eighteen hours before death. This curve (Fig. 5) showed frequent extrasystoles, a broadened QRS complex and apical notching of the maximum deflections in Leads II and III. The most striking change was the loss of the R-T interval in Lead I with the branching of the T-wave directly from the descending limb of the R-wave. In Lead III, the T-wave likewise branched directly off from the S-wave definitely below the isoelectric line.

Course.—This patient died a few days after his admission to the hospital.

Autopsy (By Dr. C. C. Garber):

Heart.—The coronary arteries of the heart were injected with an opaque mass and x-ray pictures were taken. This procedure did not give us any evidence of artery obstruction. The heart weighed 635 gm. The left ventricular wall at the apex measured 10 mm. in thickness. The right ventricular wall was 6 mm. and the left 18 mm. in thickness while the posterior wall of the left ventricle measured only 7 mm. in thickness. The valves were all intact and not unusual. The endocardium was smooth and glistening. The posterior papillary muscles were smaller than usual. The cut myocardium showed a reddish brown appearance. In the region of the apex, the muscle was thinned out and grey streaks of connective tissue were present. The posterior wall of the left ventricle in the distribution of the circumflex branch of the left coronary artery was thinned out. The section of the muscle wall in this area consisted almost entirely of connective tissue. The coronary orifices were not unusual but there were a few areas of yellow intimal thickening about them in the sinuses of Valsalva. Occasional patches of intimal thickening were noticed along the coronary vessels. The left coronary artery near its origin and also the circumflex branch were narrowed, the latter so that a small probe could not be passed into it.

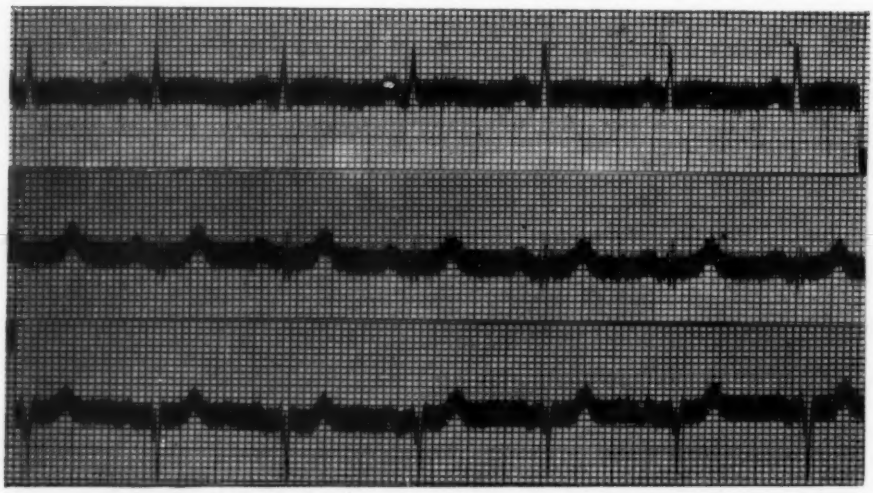

Fig. 4.—Electrocardiogram taken about seven months after attack of severe heart symptoms.

Microscopic:

Heart.—(Near the apex of the left ventricle.)

The muscle striations were poorly preserved. There was a moderate amount of diffuse scarring with replacement of atrophic muscle cells by fibrous tissue. In some of the scars there were numerous polymorphonuclears and a few wandering cells. A small area of necrosis, infiltrated by polymorphonuclears was seen. A vein in the epicardium showed a small thrombus occupying about one-third of its lumen. No bacteria were seen in the special stained sections.

(Thinned out portion of left ventricle.)

The endocardium and epicardium were slightly thickened by connective tissue. The muscle cells showed brown atrophy. In many areas the muscle cells were undergoing degeneration and atrophy, some being replaced by connective tissue. There were several areas of hemorrhage. Several capillaries contained the injection mass but several large arteries were empty.

Aorta.—The intima was thickened and partially hyalinized, the surface layers were also infiltrated by a small number of wandering cells. There was some calcification and no perivascular round-cell infiltration.

Diagnosis.—Arteriosclerosis, partial occlusion of the circumflex branch of the left coronary artery with localized atrophy and scarring of the myocardium; thrombosis of a smaller branch with infarction; myocardial degeneration.

Experimental Observations.—In 1909 Eppinger and Rothberger[7] observed that the injection of silver nitrate solution deep into the wall of the dog's heart, destroying the left ventricular muscle, caused a peculiar and changing alteration in the electrocardiogram. This change consisted of an elevation of the T-waves which branched directly off from the descending limb of the R-wave. This T-wave gradually changed, the R-T branch descending lower and lower till it approached isoelectric with finally the reappearance of the isoelectric R-T interval.

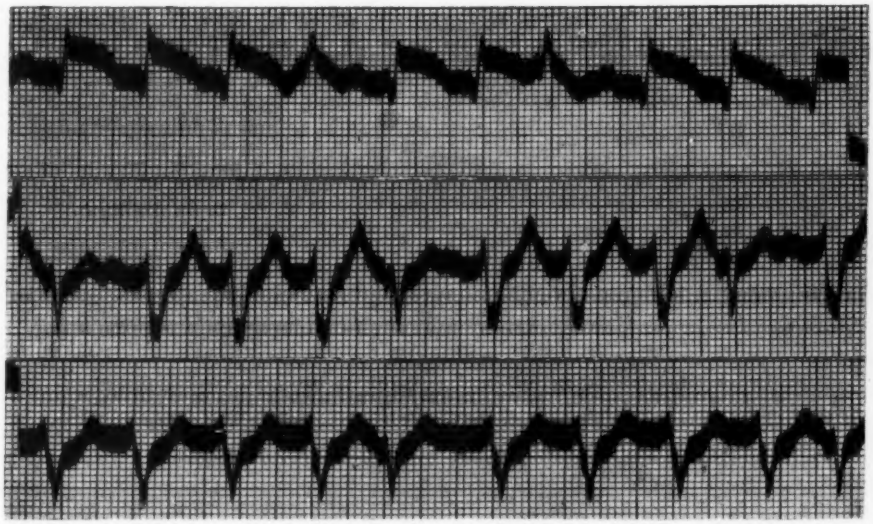

Fig. 5.—Electrocardiogram taken eighteen hours before death and apparently but a short time after the occurrence of the last and fatal heart attack.

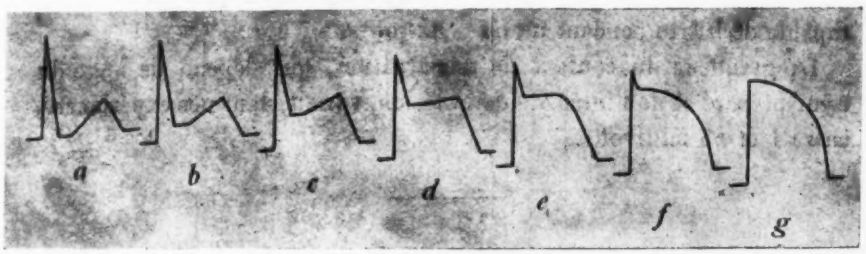

Fig. 6.—Samajloff's curves showing the action of the demarcation current produced by injury at the apex of the heart on the electrocardiogram. The figure (*g*) shows the effect of maximum apical negativity due to muscle injury. The gradual decreasing of the results of apical injury on the electrocardiogram is shown by the changes back to the normal form (*a*).

In 1918 Smith,[8] operating on dogs, ligated branches of the coronary arteries. The electrocardiograms from a large percentage of his dogs showed the T-waves branching off from the descending limb of the R-waves. The T-waves gradually approached isoelectric and finally became inverted. He repeated these experiments in 1920[9] and found that change in the T-wave was greatest in those dogs where the blood supply to the apex of the heart was most disturbed. Accordingly, he obtained the greatest changes where two or more branches of the ramus descendens and the circumflex sinistra which

supply the apical area were ligated. The ligation of arterial branches which supplied the right ventricle did not produce these changes in the electrocardiogram.

For years it has been known that the cutting of one end of a muscle bundle causes a difference of potential to arise between the cut end and the surface of the remaining uninjured portion. The injured end becomes electronegative, the surface of the uninjured portion being electropositive. Under

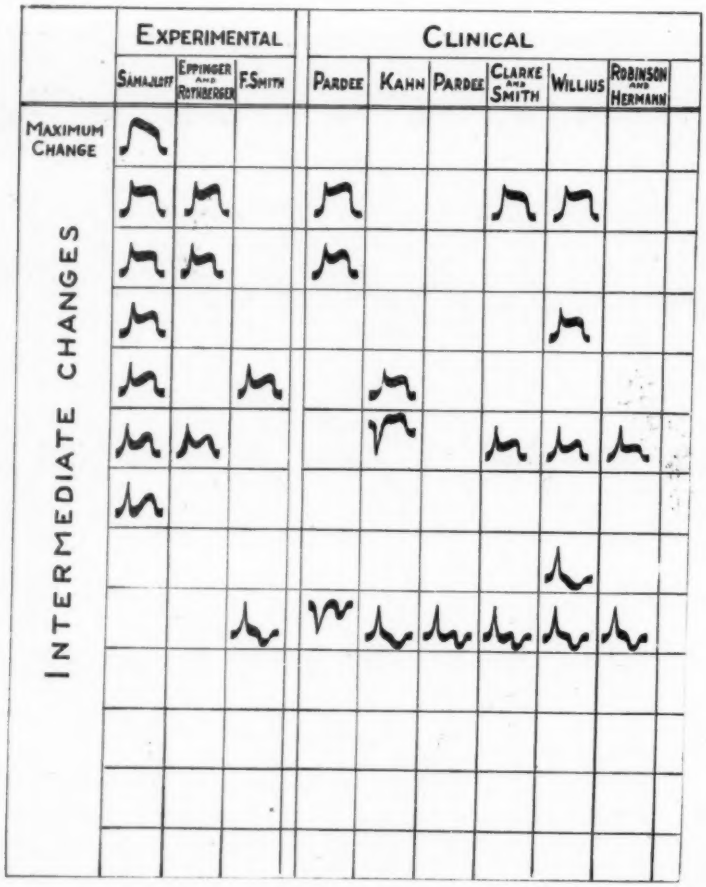

Fig. 7.—Comparison as to form of the transitional curves experimentally produced by injury to the ventricular musculature with the published curves of cases of coronary occlusion.

favorable circumstances, a current will flow from the positive portion of the muscle through a conducting medium to the injured or electronegative end of the muscle. This current, though very slight, can be detected and measured by the finer methods of precision such as by the electrocardiograph.

This physiologic fact has been extended by Samajloff[10] in working with frogs' hearts. He applied his electrodes directly on the heart at the apex and base. He found that pinching or cutting the heart muscle at its apex caused the T-wave of the electrocardiogram to be elevated and branch directly from the R-waves, the duration of the altered curve or its progressive

return to its previous normal type depending on the depth of the muscular cut, and on the age of the heart muscle.

In the frog's heart with apical injury, this peculiar alteration in the T-waves of the electrocardiogram was found by Samajloff to show a recession within five minutes, the return to normal being completed within one hour. The current set up by the difference of potential between the dead and living portions of the muscle fibers, ceased as soon as the injured fibers were entirely dead. In animals, the monophasic current derived from putting two electrodes on the heart, one on the injured and one on the uninjured surface, is temporary. The curves gradually change from the monophasic to the usual type obtained in direct leads. The progressive change in the electrocardiogram due to the interference of the demarcation current as observed by Samajloff are shown in Fig. 6.

Comparison of Clinical and Experimental Curves.—In reviewing the electrocardiograms from our own patients of coronary thrombosis, those of published cases and the mentioned experimental records, we noticed two striking similarities. The first was the same cyclic transition or variation of the tracings as time progressed and secondly the same elevation of the T-waves, branching directly off from the R- or S-wave, occurring in the true cases of coronary thrombosis and those tracings obtained by various experimental methods, all of which have included injury to the ventricular muscle. The chief difference between the clinical and experimental electrocardiograms is that the first were obtained by indirect leads whereas the experimental tracings were obtained by leads placed directly on the heart.

In Fig. 7, the experimental curves produced by the demarcation current at the apex of the frog's heart, also by tying off the coronary arteries of a dog and by injecting silver-nitrate solution into the ventricular muscle of the dog's heart are compared with those obtained from the human heart after obstruction to the coronary arteries due to disease.

This chart readily shows that the various examples of electrocardiograms which have been considered as indicating coronary thrombosis in the human subject can all find their close counterpart in the series of curves showing the effect of experimental injury to the muscle of the left ventricle.

The experimental findings of Smith by coronary artery ligation and of Eppinger and Rothberger by injecting silver-nitrate solution into the deep layers of the left ventricular muscle likewise find their counterpart in Samajloff's curve of the demarcation current produced by injury to the apex of the frog's heart. This similarity is more striking when one recalls that Smith could not produce this electrocardiographic change by obstructing those coronary branches which supply the right ventricular chamber.

This peculiar abnormality of the electrocardiogram has been observed in acute rheumatic fever by Cohn and Swift.[12] They thought it was not characteristic of the nature of the injury but rather the result of injury involving a given area. They inferred that its occurrence in rheumatic fever was due to Aschoff nodules or perhaps ischemic areas consequent upon blood vessel involvement causing injury to the muscle with subsequent electrical

disturbance. Their explanation of ischemic areas is the same as arises in coronary vessel obstruction and the "given area" which they considered involved seems on clinical and experimental grounds to be in the left ventricle and at or near the apex of the heart.

The forms of the electrocardiograms which we have presented as evidences of coronary obstruction evidently occur shortly after the injury and with the passage of time, the electrocardiogram is replaced by one not necessarily characteristic.

SUMMARY

The diagnosis of coronary thrombosis is frequently doubtful and the electrocardiograph has been shown to be of decided assistance. Numerous cardiac irregularities have been noted which are not characteristic.

The alteration of the electrocardiogram characteristic for occlusion has generally been confined to changes in the T-waves.

No definite alteration of the T-waves in coronary thrombosis has been uniformly accepted by the many writers as characteristic.

We have reported three cases with electrocardiograms, one having been confirmed by autopsy.

In experimental investigations, where injury was produced to the ventricular muscle, Eppinger and Rothberger, Smith, and Samajloff found that the T-waves of the electrocardiogram were elevated and branched directly from the R- or S-wave. They further noted that with time this elevation of the T-wave, descended to isoelectric with a reappearance of the isoelectric R-T or S-T interval.

In Samajloff's experiments he found the same change in the T-waves of the electrocardiogram due to the interference of the demarcation current between the injured area of negativity and the positively charged surface of the uninjured muscle.

The published clinical curves all find their counterpart in the series of tracings obtained by these various experimenters. The experimental curves were taken by leads directly on the heart, whereas the clinical records are from arm and leg leads.

This change in the electrocardiogram has been observed in acute rheumatic infections by Cohn and Swift. They consider it due to possible blood vessel involvement causing muscular injury with subsequent electrical disturbance.

A characteristic electrocardiographic change appears when the coronary obstruction causes injury to the left ventricle, but is not as constant when the right ventricle is involved.

The similarity of the electrocardiograms obtained by experimental injury to the ventricular muscle through various methods with electrocardiograms from clinical examples of coronary thrombosis, suggests that the acute muscular insult in both instances causes similar electrical disturbances which produce a characteristic change in the T-waves of the curves. For clinical

purposes this change consisting of elevation of the T-waves which branch directly from the R or S limb may be considered as strong presumptive evidence of coronary occlusion.

If this peculiar change is typical for coronary thrombosis in the human subject it is essentially more or less transient and soon is replaced by changes not characteristic.

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THE TOXICITIES OF LOCAL ANESTHETICS

With Special Reference to Apothesine*

By Herbert C. Hamilton, Detroit, Mich.

THE increasing use of local anesthesia has made the physician and surgeon more and more discriminating in his selection of the agent. The discovery and clinical use of cocaine by means of which minor operations could be carried out without general anesthesia is regarded as one of the epochs in the field of therapeutics. Were it not for the after effects of this valuable agent no substitute would ever have been sought. Its habit-forming effects leading to drug addiction have to a very great extent discredited cocaine. Its toxicity, while not to be disregarded, is ordinarily a less serious objection.

The long list of substitutes—eucaine, β-eucaine, holocaine, stovaine, alypin, novocaine—to mention only the outstanding ones—indicates the almost feverish interest shown in finding a satisfactory substitute for cocaine.

Of the more recently proposed local anesthetics, apothesine and butyn have been used enough to give a fair line on their availability, while isocaine, in which a propyl radical replaces the ethyl group of novocaine, is the latest one for which any data are available.

With this increasing number of local anesthetics more information is needed in order that the advantages and disadvantages of each may be fully known. Unfortunately, however, the results of tests vary greatly, indicating that the methods used and the results obtained are capable of widely different interpretation. For example, Braun[1] says, "By doubling the dose of novocaine so as to make it as effective as cocaine—novocaine has become the ideal anesthetic for injection into the tissues and has made the use of cocaine unnecessary." This was later practically verified by Closson,[2] except that, for nerve blocking, he regarded it as better than cocaine. Sollmann[3] gave novocaine a nerve-blocking value equal to cocaine but showed its value for the cornea to be one-sixteenth, for terminal anesthesia, as measured on the frog's skin, one-fourth that of cocaine. Schmitz and Loevenhart[4] showed procaine (novocaine) to be six times as effective as cocaine for nerve blocking.

Using apothesine in comparison with cocaine, Sollmann[5] at first reported it one-eighth by terminal anesthesia (intracutaneous injection into human subjects). He later revised his opinion as published by the Council on Pharmacy and Chemistry,[6] saying the value of apothesine is not better than one-half that of cocaine. By the frog skin method, Heinekamp[7] reported apothesine one-sixth as valuable as cocaine and novocaine of no value.

That competent pharmacologists should get such widely different results is evidence that accurate methods are not available by which to measure the efficiency of local anesthetics.

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Even the toxicity is variously reported. Closson[2] found cocaine eight times as toxic as novocaine, using guinea pigs subcutaneously. Hatcher and Eggleston reported a ratio of 3 to 1 by rapid intravenous injection of one large dose into cats, the actual M. L. D. being, cocaine 0.015; novocaine 0.045. Meeker and Frazer[8] found that for novocaine 0.065 was required to kill a majority of the rabbits used, by intravenous administration.

It would appear that further data on the relative toxicities of apothesine and cocaine will tend to remove some of the uncertainties regarding this important factor in local anesthesia.

When comparing drugs of similar character for efficiency and toxicity, these two effects are usually parallel. In some cases, however, such parallelism does not follow because side chains of organic compounds may vary in the intensity of the one or other of these two effects.

Apothesine is a local anesthetic one-fifth as toxic as cocaine hydrochloride[9] and equal to it for nerve blocking and terminal anesthesia.

For mucous membrane anesthesia it is one-third as effective.[10]

These results were obtained by known methods.

The toxicity data were obtained in the usual way on guinea pigs subcutaneously administered. The efficiency data for nerve blocking were obtained by action on the isolated sciatic nerve of the frog. The nerve-muscle preparation was immersed in the solution and its degree of anesthesia deduced from its response or lack of response to electrical stimulation; terminal anesthesia was tested by intracutaneous administration to four human subjects; and mucous membrane anesthesia, the Gradenwitz method,[11] by dipping one leg of a frog into the solution. The frog has its brain, medulla and heart removed. The leg is dipped into the solution for one minute, is removed and washed, and at intervals both feet are dipped into weak acid. Comparing the response to this irritation one can readily select an equally effective dilution for both the cocaine and the substance in question.

If this method is applied with almost infinite care and attention to details, the results can be regarded as trustworthy but of limited value. Heinekamp,[7] however, seems to have omitted some of the essential elements of the method, since he himself decided that they were valueless.

Ordinarily when the effective dose of a substance is far below the lethal or even mildly toxic dose, the toxicity of that substance may be disregarded. In the case of local anesthesia, however, fatalities have occurred[12] which make it necessary to investigate the causes and to consider relative advantages, having due regard for the factors of efficiency and safety.

Careful investigations of the deaths from local anesthetics reveal the fact that carelessness is often responsible[8]—carelessness in administration and even in the strength of solution. Aside from these the most important factor influencing the acute intoxication sometimes observed is the rate of absorption into the blood stream[8] (p. 389).

This fact is responsible for selecting the intravenous administration of the drug as a means of determining its toxicity. Since the reported cases of fatal poisoning are, however, so rare[12] (p. 437), and it is in most cases an

even chance that carelessness of some kind was responsible, the toxicity by this method is misleading.

I have recently compared apothesine with cocaine, not only by the intravenous administration into cats, but also by subcutaneous injection, using cats, mice and guinea pigs, and by intraperitoneal injection into mice.

The M. L. D. for mice is the same by both methods:	
Cocaine	150 mg. per kg.
Apothesine	700 " " "
ratio:	nearly 5 to 1.
The M. L. D. for guinea pigs subcutaneously:	
Cocaine	50 mg. per kg.
Apothesine	250 " " "
ratio:	5 to 1.
The M. L. D. intravenously to cats:	
Cocaine	10 mg. per kg.
Apothesine	25 " " "
ratio:	2.5 to 1.

The M. L. D. subcutaneously to cats cannot be compared on account of the rapid elimination of the apothesine. No death occurred with any dose administered. The M. L. D. of cocaine and maximum of apothesine administered were:

Cocaine	60 mg. per kg.
Apothesine	800 " " "

This is explainable by the fact that apothesine is rapidly eliminated at a rate which almost equals absorption. Eggleston and Hatcher[12] bring this point out clearly, showing that nine and one-half times the intravenous fatal dose of apothesine was administered into cats subcutaneously without producing convulsions. Further, that doses just sublethal can be administered intravenously at twenty-minute intervals almost indefinitely.

This is eloquent testimony to its safety in anesthesia.

There is, of course, always a question as to how much dependence may safely be placed upon toxicity tests carried out on animals. It is not logical to assume any relationship between the M. L. D. for any animal and that for man. The difference in susceptibility of different animals—dogs, cats, rats, pigs—indicates that similar or greater differences would be found in the lethal doses for any species and for man.

This seems to be especially true for a local anesthetic, if one accepts the occasional deaths as due to no other factor than to the anesthetic itself. Data seem to indicate that in most cases carelessness and in some cases idiosyncrasy is responsible. On occasions it is highly probable that it is a coincidence with some other responsible poisoning.

The real value of toxicity tests does not lie in the absolute M. L. D. but in the ratio of the M. L. D. of the substance in question to that of a similar substance whose clinical behavior is known.

This is a point often overlooked by pharmacologists, leading to wrong conclusions. No dose for any species of animal can safely be transferred to

man. Differences in the method of administration and susceptibility, to say nothing of the infinitely great difference in order of intelligence, make the use of the cat as the animal most nearly approaching man, a ridiculous assumption.

These data show, therefore, that apothesine is relatively only one-fifth as toxic as cocaine, except when it enters the blood stream in considerable quantity, an eventuality which can scarcely be regarded as having any bearing on the common use of a local anesthetic except in close proximity to a large blood vessel.

The purpose of using adrenalin with a local anesthetic is primarily to localize the effect by preventing absorption materially beyond the area injected, taking advantage of the well-known constricting action of adrenalin. There is, in addition to this, the strongly stimulating effect it has on the heart, an effect exactly the reverse of that of cocaine or apothesine. While this action is only slight from a subcutaneous administration, intravenously the action is prompt; it very definitely tends to offset the depressant effect of the anesthetic.[13]

There is, therefore, a double logic in associating these two powerful substances.

The extensive and satisfactory use of apothesine in anesthesia,[14] the few cases of poisoning reported, and the laboratory data showing its efficiency, low toxicity and prompt elimination in comparison with cocaine, indicate that apothesine more nearly duplicates cocaine for terminal anesthesia and nerve blocking than any other local anesthetic.[15]

TOXICITY

METHOD	SUBCUTANEOUS							INTRAVENOUS					
Reference	(9)	(2)	(4)	(14)	(16)	(17)	Average	(12)	(16)	(14)	(9)	(16)	Average
Cocaine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Novocaine	0.12	0.12	0.14		0.2		0.15	0.35	0.26				0.3
Apothesine	0.2						0.2	0.75			0.4		0.57
Butyn			1.5	2.5		3.6	2.6			1.0		1.0	1.0
Isocaine			0.3				0.3						

EFFICIENCY

METHOD	NERVE BLOCKING					TERMINAL				MUCOUS MEMBRANE		CORNEA	
Reference	(2)	(1)	(4)	(3)	(9)	(2)	(3)	(5)	(9)	(7)	(9)	(4)	(3)
Cocaine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Novocaine	1.0	0.5	6.0	1.0	1.0	1.0	1.0	1.0	0.33		0.33		0.6
Apothesine					1.0		0.12	0.5	0.33	0.16	0.33		0.12
Butyn			3.0							1.0		0.9	
Isocaine			6.0									1.4	

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LABORATORY METHODS

AN ACCURATE METHOD FOR THE ESTIMATION OF UREA IN BLOOD AND URINE BY DIRECT NESSLERIZATION*

By Joseph H. Roe, Ph.D., and Oliver J. Irish, M.S., Washington, D. C.

VARIOUS procedures[1, 2, 3] are in use at the present time for estimating the urea in protein-free blood filtrates, or untreated urine mixtures, by enzymic hydrolysis and direct nesslerization of the ammonia in the hydrolysate. These methods are all open to the serious objection that more or less turbidity develops in the nesslerized mixture, and turbid mixtures cannot be read colorimetrically with any degree of accuracy.

The turbidity obtained when nesslerizing directly urines, or tungstic acid blood filtrates, which have received urease treatment, is due to the precipitation of the mercury in the Nessler's reagent by traces of proteins, and other organic matter, in both the urease solution and the substrate. The solution of the problem of producing satisfactory colors when nesslerizing such mixtures is the removal of mercury precipitants. After an investigation of many clarifying procedures, the authors have found that the turbidity-producing substances can be removed most conveniently and satisfactorily by treatment with calcium phosphate in an alkaline medium. This procedure consists of alkalinizing the mixture, adding a little calcium nitrate, which precipitates the phosphate previously added as a buffer for the urease digestion, mixing thoroughly, and centrifuging. The calcium phosphate adsorbs the objectionable organic matter, and the clear supernatant solution obtained after centrifuging is decanted and nesslerized. The color obtained in a solution filtered free from mercury precipitants by this procedure compares excellently with that produced by a pure ammonium salt solution when nesslerized.

PROCEDURE FOR BLOOD

Pipette 2 c.c. of blood into a small Erlenmeyer flask and add 1 c.c. of potent urease solution and 2 drops of Folin's buffer phosphate mixture. Cork and place at 50° C. for fifteen minutes. After enzymic digestion, the blood is treated to produce a protein-free filtrate according to the regular Folin-Wu procedure. Add 13 c.c. of distilled water, 2 c.c. of sodium tungstate, and 2 c.c. of two-thirds normal sulphuric acid; mix thoroughly, allow to stand for two or three minutes until the typical "chocolate brown" color appears, then filter through an ammonia-free 9 cm. folded filter paper. (Fil-

*From the Department of Chemistry George Washington University Medical School. Received for publication, April 28, 1926.

ter paper around 9 cm. in size is desirable to get the most filtrate.) Place 10 c.c. of the tungstic acid filtrate in a 15 c.c. conical centrifuge tube, add 0.5 c.c. of 20 per cent sodium hydroxide, 1 drop of buffer phosphate mixture (to insure precipitation of calcium), 2 drops of 10 per cent calcium nitrate, and stir the precipitated calcium phosphate with a glass stirring rod until it is distributed uniformly through the mixture. Centrifuge for two minutes, then decant the supernatant liquid into a 25 c.c. graduated cylinder. Wash down the sides of the centrifuge tube with 2 c.c. of 1 per cent sodium hydroxide, and again mix uniformly the calcium phosphate by breaking up the mat in the bottom of the tube and stirring with a glass rod. Centrifuge for two minutes, then decant the supernatant fluid into the original mixture. Add 3 c.c. of Nessler's reagent, and make up to 15 c.c. with distilled water. This will be the correct volume for color comparison if the blood has a normal urea content. If an increased urea is encountered, the mixture is diluted to a volume that will approximately match the standard by adding distilled water and Nessler's reagent, the amount of the latter introduced being one-fifth the final volume of the mixture.

Prepare a nesslerized standard nitrogen solution as follows: In a 100 c.c. glass-stoppered graduated cylinder place 5 c.c. of standard ammonium sulphate solution containing 1 mg. of nitrogen; add distilled water to the 50 c.c. mark, Nessler's reagent to the 70 c.c. mark, then distilled water to 100 c.c., and mix thoroughly. Compare in a colorimeter in the usual manner.

CALCULATION

$$\frac{\text{Reading Standard}}{\text{Reading Unknown}} \times 1 \times \frac{\text{Dilution Unknown}}{100} \times 100 = \text{Mg. Urea N per 100 c.c. Blood.}$$

The step where the centrifuge tube is washed with 2 c.c. of 1 per cent sodium hydroxide may be omitted in clinical work without appreciable loss of accuracy by introducing a correction factor for the amount of ammonia in the fluid left in the tube after the first decantation. We have found that the average amount of water left in a cleanly draining tube after decantation is around 0.5 c.c. This is about one-twentieth of the solution being analyzed, and will therefore require a 5 per cent correction factor. This correction is conveniently introduced by substituting 105 for 100, the last factor in the above formula for calculation.

PROCEDURE FOR URINE

Transfer 5 c.c. of urine to a 50 c.c. volumetric flask, make up to the mark with distilled water, and mix thoroughly. Pipette 1 c.c. of this mixture into a 15 c.c. conical centrifuge tube, add 1 c.c. of potent urease solution, 2 drops of Folin's buffer phosphate mixture, and place the tube in a water-bath at 50° C. for fifteen minutes. At the completion of the enzymic digestion remove the tube and add 8 c.c. of ammonia-free water, 1 c.c. of 10 per cent sodium hydroxide, and 2 drops of 10 per cent calcium nitrate solution. Stir the mixture with a glass stirring rod until the calcium phosphate precipitate is uniformly distributed, then centrifuge for two minutes. After centrifugation,

decant the clear supernatant liquid into a 100 c.c. glass-stoppered graduated cylinder. To remove the ammonia adherent to the sides of the centrifuge tube add 10 c.c. of 1 per cent sodium hydroxide, break up the mat of calcium phosphate in the bottom of the tube with a glass stirring rod, centrifuge as before, and decant the supernatant washings into the original mixture. Add distilled water to the 40 c.c. mark, then 10 c.c. of Nessler's reagent, and mix thoroughly. If the color obtained is too deep for favorable comparison, the mixture is diluted to a desirable volume by adding more distilled water and Nessler's reagent, always introducing into the solution an amount of Nessler's reagent equal to one-fifth the final volume. Prepare a standard as directed above under *Procedure for Blood*, and compare in a colorimeter in the usual manner.

CALCULATION

$$\frac{\text{Reading Standard}}{\text{Reading Unknown}} \times 0.001 \times \frac{\text{Dilution Unknown}}{100} \times \frac{\text{24 hr. Volume in c.c.}}{0.1} = \text{Grams Urea N} + \text{Ammonia N per 24-hr. sample of Urine.}$$

To obtain Urea N, subtract Ammonia N determined separately by the permutit or other methods.

EXPERIMENTAL AND DISCUSSION

This procedure was checked against the Myers[4] urease and aeration method for urea estimation upon pure urea solutions and upon numerous blood and urine samples. Twenty-four samples of a pure urea solution of

TABLE I

SAMPLE NO.	AERATION METHOD GRAMS UREA N PER 100 C.C. URINE	AUTHORS' METHOD GRAMS UREA N PER 100 C.C. URINE
1	0.55	0.55
2	0.87	1.03
3	1.07	1.13
4	1.20	1.54
5	1.33	1.33
6	1.25	1.25
7	1.21	1.35
8	1.57	1.80

TABLE II

SAMPLE NO.	AERATION METHOD MG. UREA N PER 100 C.C. BLOOD	AUTHORS' METHOD MG. UREA N PER 100 C.C. BLOOD
1	10.5	10.5
2	12.8	13.5
3	12.8	12.7
4	13.6	14.8
5	15.2	14.7
6	32.7	35.7
7	47.5	48.0
8	69.0	68.5
9	128.2	127.5
10	222.0	235.0

known value were treated with the same enzyme solution under identical conditions, and the urea nitrogen in one-half of these was determined by the aeration procedure, and in the other half the calcium phosphate clarification method was used. The results obtained by the latter procedure were more consistent, and averaged 5.2 per cent higher than those obtained by the aeration method. We have carried out many parallel determinations upon blood and urine samples, using the same two procedures, and usually have obtained close comparisons, but the general tendency has been to get higher results by direct nesslerization after calcium phosphate clarification than when the ammonia is removed by aeration to a separate tube before being nesslerized. Typical protocols are shown in Tables I and II. Granting that the reagents used are ammonia-free, it is obvious that the recoveries by direct nesslerization, when clear colors are obtained, are more correct, because additional steps in a quantitative method, especially uncertain processes like aeration or distillation, increase the possibilities of error. We, therefore, claim that this method is not only a much easier and shorter procedure, but that it is also a more accurate method than those involving aeration or distillation.

SUMMARY

We claim for this method of determining urea nitrogen in blood and urine the following advantages:

1. It always gives clear colors for comparison, and is therefore superior to the usual direct nesslerization procedures in which more or less turbidity develops.

2. It is highly accurate. Since the ammonia is nesslerized in the original solution, an important source of error is eliminated, which always exists in the methods that involve the removal of ammonia to a separate container. It gives consistently more accurate results than the aeration procedure, because it does not permit any losses that may result from passing air too rapidly, or from discontinuing aeration before all the ammonia is removed. Neither is it subject to the technical difficulties and inaccuracies of micro-distillation procedures.

3. It is a great saving of time and labor. An accurate analysis can be obtained in less than thirty minutes.

4. As the technic is less difficult and requires less complicated apparatus, this method is more adaptable to the physician's office practice.

5. It is a valuable asset for teaching the determination of urea nitrogen to large classes of students where the use of aeration or distillation apparatus is an administrative problem.

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A SIMPLE HYDROGEN ELECTRODE FOR USE IN BIOCHEMISTRY*

By Allen D. Garrison, Ph.D., Henry O. Nicholas, Ph.D., and Joe G. Pasternack, B.A., Houston, Texas

THE methods most widely used in studying the P_H of biologic solutions are the electrometric methods, based upon measurement of an electric potential, and the colorimetric or indicator methods, which utilize indicators whose solutions give color effects dependent upon P_H. Although the former

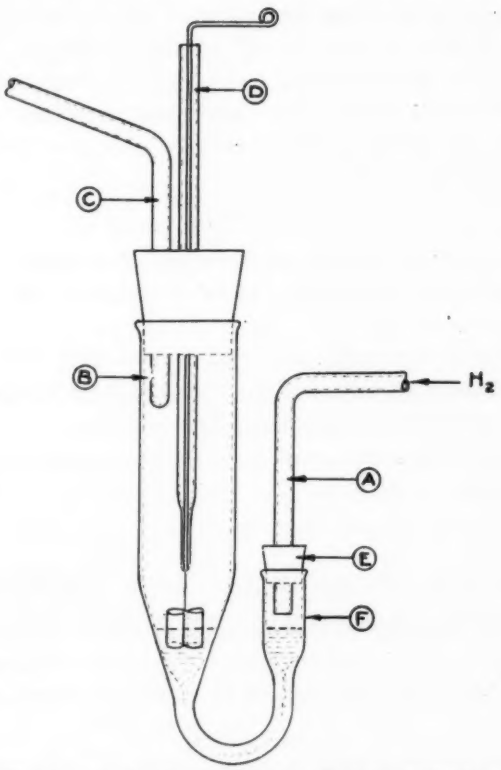

Fig. 1.—A simple capillary hydrogen electrode.

is the method of ultimate reference, electrometric P_H determinations are avoided because of inconvenience, especially in the type of hydrogen electrode that will be best adapted for the particular work in hand.

With the electrode herein described, P_H determinations can be made on *less than one c.c. of liquid whether clear, colored, or turbid, with an accuracy of .03 P_H, the experiment taking less than five minutes.* This form of electrode has been found very convenient for biologic purposes, as in the adjustment of "reactions" of culture media, the determination of P_H of whole blood, serum, and numerous other solutions.

*From the Department of Biochemistry, Rice Institute, Houston, Texas.
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This hydrogen electrode is very easily made and requires nothing not ordinarily found in a chemical or biologic laboratory. In its preparation either a simple adapter, such as is commonly used in ordinary distillations, or a short piece of pyrex glass tubing about 15 mm. in diameter is used.

Hydrogen from a generator enters at *A*. The gas rises through the liquid saturating the liquid as it passes through and carrying out the air with it through the holes at *B*. After two to four minutes of gentle bubbling, the tube *C* is pulled up through the rubber stopper so that the holes at *B* become closed, leaving an atmosphere of hydrogen over the liquid. The platinum blacked electrode is now adjusted by means of the glass rod *D*, so that half of the platinum is immersed in the solution. The stopper *E* is now removed and the tip of the arm of the standard calomel cell is immersed slightly below the surface of the liquid in the arm *F* of the hydrogen electrode. In the manipulation care must be taken not to tip the hydrogen electrode about too much or air bubbles will enter and break the capillary column of liquid. This, however, does not destroy the determination provided the column can be reunited immediately.

SUMMARY

1. A modified form of hydrogen electrode requiring less than 1 c.c. of liquid for examination is described. The liquid may be clear, colored, or turbid.

2. The electrode is especially adapted to use with biochemical solutions.

3. The time required to saturate the solution with hydrogen and to bring the electrode to equilibrium is less than five minutes.

Thanks are due to the laboratory staff of the Hermann Hospital for the blood samples used in this investigation.

A MODIFICATION OF GOODPASTURE'S TECHNIC FOR THE PEROXIDASE REACTION IN BLOOD SMEARS*

By Dean N. Beacom, M.D., Denver, Colorado

IN 1919 Goodpasture[1] described a stain which is used to demonstrate the peroxidase reaction within certain leucocytes. This is a great aid in differentiating leucemias of the lymphatic and the myelogenous types.

The stain which he prepared has the following composition:

Alcohol, 95 per cent	100.00 c.c.
Sodium nitroprusside	0.05 gm.
Benzidine, C. P. (Harmer)	0.05 gm.
Basic fuchsin	0.05 gm.
Hydrogen peroxide	0.5 c.c.

I have found this stain to be very satisfactory except for the fact that it is not stable. The hydrogen peroxide deteriorates very rapidly, and a

*From the Department of Clinical Pathology, University of Colorado School of Medicine.

staining solution which gives good results when fresh cannot be depended upon a few days later.

It was learned by experience that when this stain ceased to bring out the granules, the addition of the proper amount of hydrogen peroxide would restore the solution to its former potency. Subsequent additions, however, were valueless.

Because of these defects a modification of Goodpasture's stain was devised which gives good results for about eight months. After this period the granules in the positively reacting cells may still be seen, but they are not nearly as distinct as they should be.

The formula which I have found to give satisfaction is as follows:

Alcohol, 95 per cent	100.00 c.c.
Sodium nitroprusside	0.05 gm.
Benzidine, C. P.	0.05 gm.
Basic fuchsin	0.10 gm.

It will be seen that in this formula hydrogen peroxide is omitted, and the amount of the fuchsin is doubled. I have found, as mentioned by Todd,[2] that a clearer picture may be obtained by increasing the amount of fuchsin. This probably is due to a difference in the strength of the dye.

When it is desired to stain the blood smear, a 1:200 dilution of hydrogen peroxide is made in distilled water. This gives the same concentration as in Goodpasture's stain. Without previous fixation a noted quantity of the staining fluid is applied to the film. After one minute the same number of drops of the aqueous hydrogen peroxide solution is added and allowed to act from three to six minutes, depending upon the stain and upon the depth of staining desired. The preparation is then rinsed, dried, and examined. By this method the granules stand out very clearly in the cells which give the peroxidase reaction.

The hydrogen peroxide dilution keeps well for some weeks; but because such a small amount is needed, it is probably just as well to make it up fresh each time since the dilution is easily made by counting the drops from a medicine dropper.

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A MODIFICATION OF THE THALHIMER APPARATUS FOR THE SLOW INTRAVENOUS INJECTION OF GLUCOSE AND SALINE SOLUTIONS*

By George T. Pack, B.Sc., M.D., University, Ala.

IN THE treatment of severe postoperative acidosis or severe vomiting of pregnancy, Thalhimer has developed a method of treatment that is quite efficacious. It depends upon the slow intravenous injection of 1000 c.c. of a sterile 10 per cent glucose solution, followed by insulin hypodermically.[1] The time consumed in giving this amount is from four to five hours; the rate

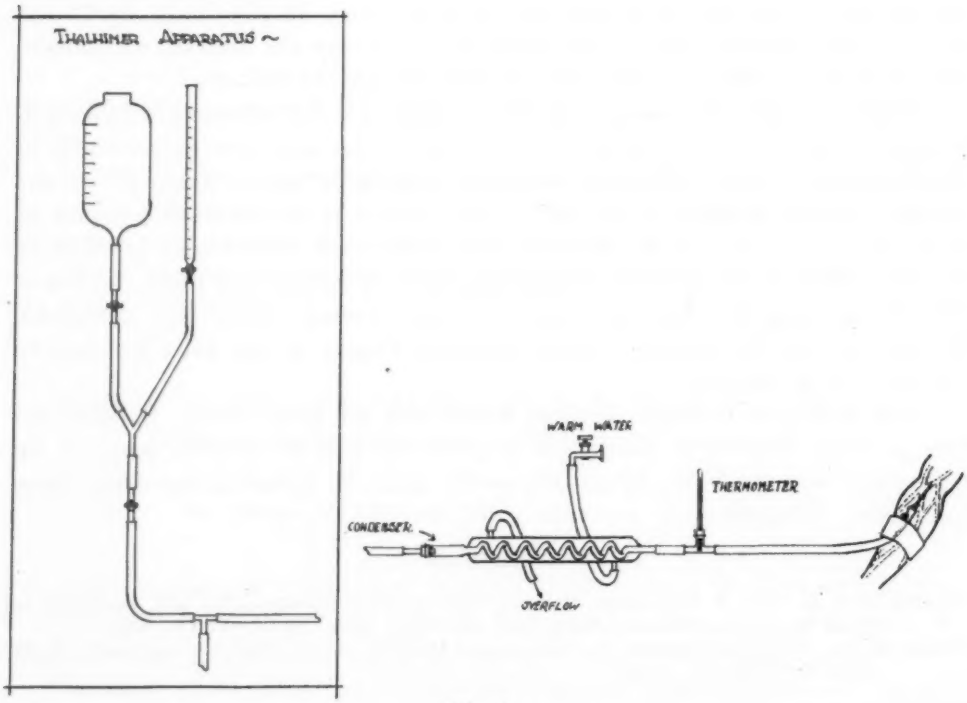

Fig. 1.

of introduction is controlled by reading the changing fluid levels in a graduated burette, fed at intervals from a main reservoir.[2]

This rate of flow is so slow that the solution must inevitably cool before it enters the vein. To enclose the reservoir with suspended hot water bags will not greatly obviate this defect. The temperature of the solution injected

*From the School of Medicine, The University of Alabama,
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should be at least 105 degrees F. (41 degrees C.). Even when the rate of flow is as rapid as 500 c.c. in ten minutes the temperature of the fluid will be lowered two degrees as it flows from the container to the vein of the patient. If the fluid is administered at the temperature of 115 degrees F. (46 degrees C.), the heat will exert a stimulating effect on the circulation and the sugar will be more readily utilized.

A method is illustrated for the maintenance of a high and equable temperature of the fluid. An ordinary glass condenser (sterile) is interposed in the circuit together with a thermometric attachment. The outer chamber of the spiral condenser is connected with slowly running hot water, the source of which is either a convenient hot water tap or a funnel to which hot water is added as needed. The thermometer is sterilized by immersion in a 1 to 500 mercury bichloride solution and inserted in the upright limb of a glass T-tube.

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AN IMPROVED COLONY COUNTING APPARATUS*

By T. C. Buck and Jos. C. Swenarton, Baltimore, Md.

IN ORDER that one may be sure that he is counting the colonies on Petri plates under uniform conditions, it is essential that the illumination be standardized and, therefore, artificial.

The only colony counting apparatus we know of which is designed for use with artificial light is the one described by Stewart in the *Journal of Medical Research,* January, 1906. This apparatus, while very useful for some purposes, has certain drawbacks for use in the routine counting of many plates.

The chief objection to the apparatus for this work is that too much light is transmitted through the plate, thereby making transparent colonies difficult to see. It is also unsatisfactory to have the ruled guide plate above the colonies, because, on crowded plates, it is impossible accurately to keep track of which colonies have been counted and which have not been counted.

It was to overcome these objections that we undertook the construction of the apparatus described here. The design, which is shown in the accompanying cut, was evolved in a purely empirical way. That is, from our experience with the Stewart counting apparatus and the ordinary ruled background apparatus we were able to get ideas as to how the apparatus

*From the Bureau of Bacteriology, Health Department, Baltimore, Md.
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might be improved. Thus, after trying various combinations, we finally produced the design illustrated.

While our method of procedure was empirical, we believe that the principle involved is one of the correct combination of transmitted and reflected light. In this apparatus we get enough transmitted light to illuminate the agar, while the colonies are seen by reflected light. In the Stewart counting apparatus not only the agar becomes transparent but the colonies also. The result of the combination developed in this apparatus is that all colonies can be distinguished easily without eye strain.

DESCRIPTION

The apparatus consists of a box 9 × 11 × 2⅜ inches. This is fitted with a blue background plate. On top of the blue background plate is placed a

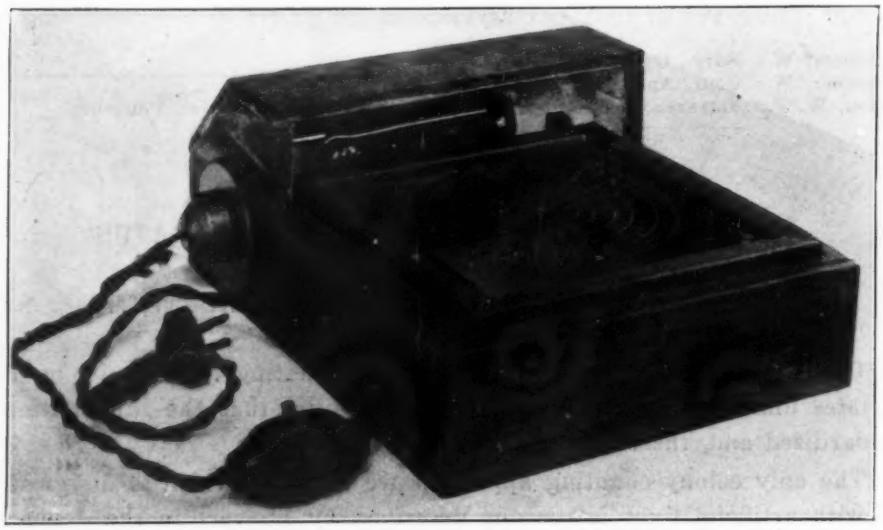

Fig. 1.

ruled Jeffer plate, which in turn is covered with a piece of plate glass. An electric light bulb furnishes the illumination. The inside of the box is painted white, and in the bottom under the glass plates is a reflector plate of glazed porcelain. The electric light is a 25 watt elongated bulb and is so placed that the longitudinal axis of the bulb is in the same plane as the middle glass plate. The bulb is covered with a reflecting hood which has a surface built at the proper angle to reflect the light onto the colonies. This hood also protects the eyes from the light. In order to make the transmitted illumination uniform, the end of the box away from the light is sloped so that some of the light is reflected back onto the glazed porcelain reflector. This tends to illuminate this reflector uniformly.

In making colony counts, the Petri dish is placed on the plate glass which covers the Jeffer plate and the colonies viewed through the regulation hand lens.

SUMMARY

A colony counting apparatus is described and illustrated which is superior to any on the market for the reasons:

1. It provides for a uniform illumination.

2. The colonies are illuminated in such a manner that they can easily be distinguished without eye strain.

3. It is simple in construction and includes no unnecessary parts which might interfere with the rapidity with which it can be used.

A DENTAL CONTRIBUTION TO THE BASAL METABOLISM TEST*

By John Bell Williams, D.D.S., and Margaret Nolting, M.D.
Richmond, Va.

MANY patients, because of the absence of natural or artificial teeth, or because of the abnormality of the alveolar ridges, present a problem to the operator in securing on them a correct basal metabolism rate. Such patients are unable to take a proper grip on the mouthpiece of the metabolism apparatus and a leakage results.

Fig. 1.—Dental impression trays and impression material.

In our hands mouthpieces in general usage are not altogether satisfactory. With this in mind we sought a mouthpiece which would minimize leakage. Our appliance is based upon certain well-known dental principles. It is inexpensive and easily constructed, and with its aid patients can hold a mouthpiece with ease and comparative comfort.

The first step in constructing our appliance is shown in Fig. 1 which exhibits the typical dental impression trays, together with a hard impression material composed of stearin, gum dammar and powdered soapstone colored with carmine. This impression compound which is softened by mild heat is used for securing a correct impression of the alveolar ridges and hard palate. The impression is then removed from the mouth and tray (Fig. 2). The impression is returned, without the trays, to the mouth. Sufficient im-

*From the McGuire Clinic, Richmond, Va.

Fig. 2.—Impression material removed from the mouth and trays showing imprint of alveolar ridges and hard palate.

Fig. 3.—The finished appliance mounted on an articular.

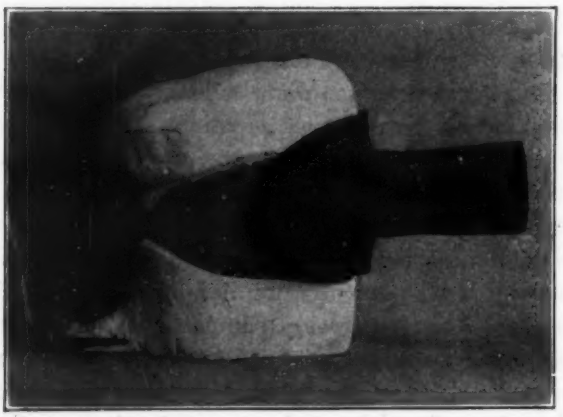

Fig. 4.—Showing the appliance and mouthpiece in proper relation.

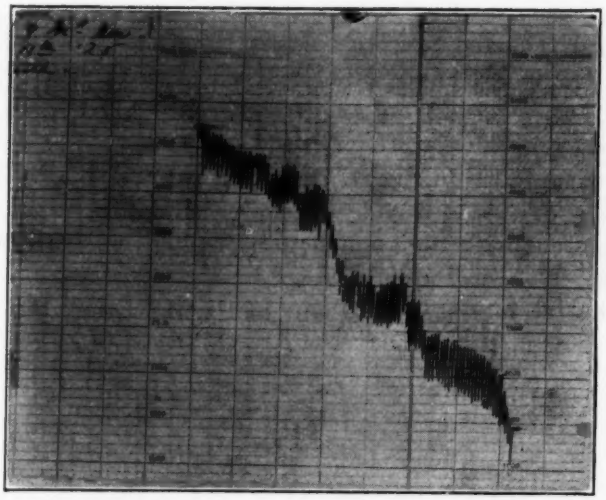

Fig. 5.—Shows an unsatisfactory tracing made with the mouthpiece retained by adhesive plaster.

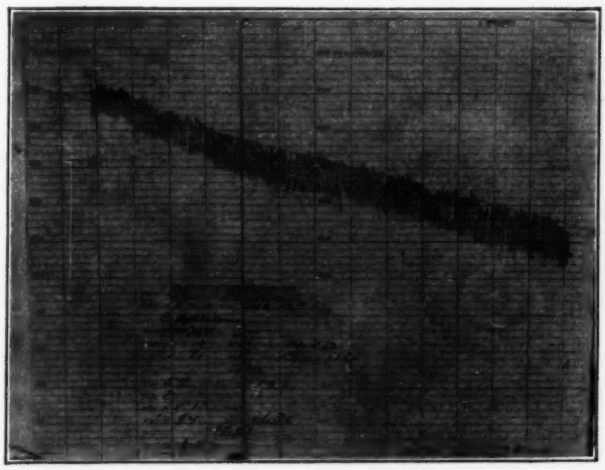

Fig. 6.—Shows a satisfactory tracing made with our appliance.

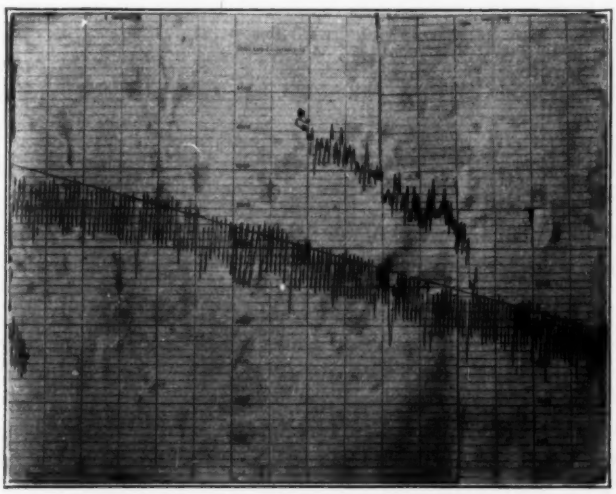

Fig. 7.—Shows an unsatisfactory tracing made without the appliance side by side with a satisfactory tracing made with the appliance.

pression material is added over the ridges in order to provide a breathing space in front and to seal them with the jaws in their proper relation. The impression is then mounted on an articulation. The ridges are serrated to grasp the mouthpiece (Fig. 3). The completed appliance with the mouthpiece in position is shown in Fig. 4. Fig. 5 shows a tracing with the mouthpiece retained in the usual way by means of adhesives. The tracing made for this same patient with the appliance in position is shown in Fig. 6. The value of this appliance is forcibly shown in Fig. 7, where one may compare side by side the tracings made without and with the aid of the appliance.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Kusama, S.: On the Etiology of Typhus Fever and Measles. Japan Med. World, Nov. 15, 1925, v, No. 2, 309.

Kusama has been investigating this subject since an outbreak of typhus occurred in Tokyo in 1914.

Japanese monkeys which had contracted the disease by injection of the passage virus of typhus were bled to death and the kidneys removed aseptically.

A small portion of the kidney was placed in tubes containing 10 c.c. of ascitic broth and incubated at 37°C. for two to three weeks.

The medium consisted of one part of ascitic fluid to four parts of broth, the P_H being adjusted to 7.0.

In successful culture the tubes show turbidity in 10 to 14 days.

The organism thus cultivated is a bacillus 0.9 to 1.8 micra long and 0.4 to 0.5 micron in width. The ends may be sharp or dull, and the organism straight or slightly curved.

There may be perceptible pleomorphism.

Fresh cultures are stained with difficulty. In older, giessa-stained cultures the body of the organism is light blue and contains chromatic granules. The organism is gram-positive and nonacid-fast.

Subcultures are difficult at first but growth occurs later on various media, Loeffler's serum and potato.

Inoculation of this organism into monkeys produced a reaction somewhat similar, but less prolonged, to that following the injection of the passage virus of typhus. The histologic changes were also similar to those seen in experimental typhus in monkeys.

Cross immunity tests are recorded as positive by Kusama. Agglutinins, opsonins, or complement-fixing bodies were not produced by the organism.

After intraperitoneal inoculation the organism could be demonstrated for five days and then disappeared. Later, when the onset occurred, the organism could be recovered from the kidneys.

Using an identical technic, another organism was recovered from monkeys inoculated with blood from cases of measles.

It is a gram-positive bacillus, 1.2 to 2.3 micra long and 0.5 to 0.7 micron wide and morphologically very similar to the organism above described. It grows only on liquid media and is anaerobic; older cultures will grow aerobically on both liquid and solid media. It is chromogenic (pink).

It is apparently nonpathogenic and Kusama is attempting to show its relationship to measles by immunologic methods.

Leuci, T.: The Hematoclastic Wassermann Reaction. Rev. Assn. Med. Argentina, October, 1925, xxxviii, 415.

Leuci investigated the theory advanced by Bancin in 1923 with reference to the reacting substances producing a positive Wassermann reaction, namely, that these were in part retained within the blood cells.

Bancin tested serum into which hemoglobin had been liberated by breaking up the clot.

Leuci collected blood in tubes containing glass beads which served to guard against coagulation, to defibrinate the blood, to mechanically rupture the corpuscles, and to obtain plasma instead of serum.

Leuci believes that the procedure increases the sensitivity of the complement-fixation reaction.

Drucker, P., and Allen, G. E.: A Simple Method for Obtaining Cutaneous (Capillary) Blood from Infants and Adults for Colorimetric P_H Determination. Jour. Biol. Chem., May, 1925, lxiv, No. 1, p. 221.

Details of Technic:

The knife, a cataract knife 2 x 30 mm. must be razor sharp. The incision is about 5 mm. deep and 2 mm. wide.

The tubes are clear glass 13 x 54 mm.

The indicator NaCl solution is prepared from 0.9 per cent NaCl from distilled water. It is desirable, but not necessary, to have this free from CO_2. About 45 c.c. are placed in a 50 c.c. measuring flask and *exactly* 0.54 c.c. of 0.06 per cent phenol red solution added.

One to three drops of 0.02 N NaOH are added to make the reaction P_H 7.4 to 7.6 and the solution diluted to 50 c.c. with NaCl solution.

(The saline solution must contain $\frac{54}{50}$ as much indicator as the standard, hence the 0.54 c.c. of indicator to allow for the 0.4 c.c. of blood.)

The standard solutions are prepared from Sorensen's phosphate at 0.05 P_H intervals, using 0.01 c.c. of the same indicator per 10 c.c. of standard solution.

Phenol red stock solution: 0.1 gm. dissolved in 5.7 c.c. of 0.05 N NaOH added to about 80 c.c. of distilled water, boiled, diluted to 100 c.c. and filtered.

It is best to determine, for each lot of phenol red, what concentration gives the best difference in color.

Paraffin oil: Density 0.885.

The glass apparatus is best cleaned in sulphuric-bichromate solution.

Method:

Infants: Immerse the entire foot in hot water (45 to 50° C.), until a good hyperemia occurs.

Remove and dry with ether. Dip the heel into an ordinary glass funnel about 55 mm. in diameter, closed at the bottom of the V, and filled with paraffin oil.

Make a stab incision under the surface of the oil on the under side of the heel somewhat to one side of the midline.

Allow blood to collect under oil. With a 1 c.c. pipette graduated in 0.01 c.c. draw up 0.2 to 0.3 c.c. of oil and then 0.8 c.c. of blood.

Transfer the blood to a comparison tube containing 5 c.c. of 0.9 per cent NaCl, overlaid with oil, and another 0.4 c.c. to a tube of NaCl—phenol red solution, also overlaid with oil.

Stopper the tubes with a one-hole stopper so that all the oil is expressed, and close the hole with a glass plug.

Centrifuge 2 to 5 minutes and compare with the standards.

Adults: Specimen collected from ear or finger as described above.

Reading must be made within one-half hour.

Read the temperature of the diluted plasma.

The P_H at 38°C. is then calculated by the following formula:

$$P_H\ 38° = P_H \text{ color } T° \text{ plus } 0.01\ (t - 20°) - 0.26$$

The 0.26 is Cullen's constant for human plasma plus the correction for loss of CO_2 in oil of 0.03 to 0.04 P_H. The 0.01 $(t - 20°)$ is the correction for change in room temperature.

Bibb, L. B.: Long Tube Method of Cultivating Organisms, with Observations on Mobile Colonies in Liquid Medium. Jour. Bact., November, 1926, x, No. 6, p. 561.

The method devised by Bibb obviates mechanical and other disturbance incident to transfer and affords a continuous view of the reaction of bacteria to environment.

Ordinary glass tubing of 3 to 5 mm. outside diameter was autoclaved in 5 foot lengths which were sealed end to end in a blast lamp until a 30 foot tube was obtained.

Under aseptic precautions, and avoiding air bubbles, nutrient broth (P_H7.6) was aspirated into the tube. One end of the tube was then sealed and the other cut off one inch longer than the column of broth.

One c.c. of the culture to be studied was then emulsified near the open end of the tube which was left in a horizontal position at room temperature. B. coli from 24-hour slants was thus studied.

The progress of the bacterial growth along the tube can be followed with the naked eye, a visible turbidity preceding by about ½ inch the most advanced bacilli.

The rate of progress was uniform and averaged about 4 feet per day or 5 cm. per hour.

The turbidity was opaque at the head of the column and when viewed in profile appeared as one or two triangular areas of dense, whitish turbidity separated by a streak of relatively clear medium, transverse to the tube, and about 5 mm. wide.

About 3 to 5 cm. in the rear of the head of the column appeared a second triangular turbidity, sometimes bifid or double. Between these two areas was a segment of clear medium 3 to 5 cm. long.

The characteristics of these turbid areas varied somewhat with different cultures but was the same for the same culture.

Bacilli of certain strains regularly formed mobile colonies of characteristic shape. If these were disturbed by agitation, they again formed into their usual shape in a few minutes.

The method furnishes a practical means for observing the effect of different substances or influences on bacterial growth and activity.

The paper is illustrated.

Pelouze, P. S., and Viteri, L. E.: A New Medium for Gonococcus Culture. Jour. Am. Med. Assn., March 6, 1926, lxxxvi, 684.

The authors maintain that much of the difficulty experienced in culturing the gonococcus and, perhaps, some of the cultural characteristics attributable to the organism, are due to lack of a suitable medium.

On the medium described below gonococcus culture is declared to be as easy as other ordinary routine cultures, the organism growing indefinitely on subcultures and retaining its vitality for at least one month.

The method of preparation follows:

A calf's brain, weighing approximately 500 gm., is forced through a wide-meshed gauze into 500 c.c. of distilled water and placed in the ice box for twenty-four hours. It is then filtered several times through cotton of varying degrees of compactness. The resultant fluid is turbid, no matter how often it is filtered. To this is added 0.5 per cent of acid sodium phosphate and 1 per cent of peptone. It can now be autoclaved at 15 pounds pressure for twenty minutes and kept as stock, or the final steps for its completion can be carried out.

To complete the medium it is necessary simply to add one part of the brain bouillon to three parts of standard 2.5 per cent agar medium made from veal broth, with the addition of 0.5 per cent of sodium chloride and 1 per cent of peptone. It should then be adjusted to a P_H of 7.8 which allows for a reduction of two points in autoclaving, 7.6 being the desired end-point.

The medium is then tubed, autoclaved and slanted. After it solidifies, the usual cotton plug is replaced by a sterile rubber cork to retain the water of condensation, and the medium keeps indefinitely.

When it is completed and cooled, some flocculation is present in the butt of the tube. This can easily be removed if the medium in bulk is placed in the autoclave, quickly brought to 15 pounds pressure, removed, filtered, tubed and replaced in the autoclave for the completion of sterilization. While this improves its appearance, it seems to make some change in it which causes more scanty growths; consequently, the authors have not made use of it, preferring a good medium to a pretty one.

Tozerski, E.: A Favorable Synthetic Medium for the Development of the Bacteriophage Element. Compt. rend. Soc. Biol., Nov. 27, 1925, xcii, 1285.

The following formula was devised for the purpose indicated:

Double distilled water	1000 c.c.
Succinate of ammonia	6 gm.
Saccharose	40 gm.
Potassium sulphate	1 gm.
Magnesium sulphate	1 gm.
Disodium phosphate	0.5 gm.

The growth of bacteriophage on this medium was very satisfactory.

Tanner, F. W., Devereux, E. D., and Higgins, F. M.: The Multiplication of Yeast and Yeastlike Fungi in Synthetic Solutions. Jour. Bacteriol., January, 1926, xi, No. 1, p. 45.

Fulmer and Nelson's medium F was found satisfactory for the purpose. The formula follows:

Ammonium chloride	0.188 gm.
Calcium chloride	0.100 gm.
Sucrose	10.0 gm.
Dibasic potassium phosphate	0.10 gm.
Dextrin	0.60 gm.
Distilled water	100.0 c.c.

The ammonium chloride content is adjusted for incubation at 38° C. The flocculent precipitate of calcium phosphate (?) serves, through its disappearance, as an evidence of growth. Growth occurs in this medium in the absence of "bios."

Damon, S. R.: A Note on the Spirochetae of Termites. Jour. Bacteriol., January, 1926, xi, 36.

The following technic was used for the demonstration of spirochetae:

1. Emulsify the gut contents of the termite in a drop of 0.4 per cent saline solution and expose to the fumes of osmic acid for 30 seconds.
2. Dry in air or by very gentle heating.
3. Flood the slide with a 1:5 solution of 50 per cent carbolfuchsin and 50 per cent anilin gentian violet. Stain 60 seconds.
4. Wash in water, dry, and examine.

The background is light pink and the organisms much darker as they take the gentian violet.

The forms seen are described and illustrated in six microphotographs.

Elman, R., and McMaster, P. D.: Studies on Urobilin Physiology and Pathology. IV. Urobilin and the Damaged Liver. Jour. Exper. Med., July, 1925, xliii, No. 1, p. 99.

A continuation of studies on dogs upon this subject, this paper being concerned with the sequelae of direct injury to the hepatic cells or obstruction to the ducts.

Previous studies have shown that in the dog urobilin is formed only in the intestinal tract.

When a total bile loss was produced through hepatic injury (chloroform or biliary obstruction) and intubation of the common duct, bile and feces remained urobilin-free for as long as four and one-half months, the total period of observation in ten animals.

In eight animals in which liver injury was induced by chloroform, phosphorous, or toluylene-diamine, but only a fraction of the bile was lost by intubation of a small hepatic duct, most of the bile thus flowing to the intestine, urobilin was a regular constituent of the

bile and stool. When the bile constituents are prevented from reaching the intestine but accumulate within the body, as occurs during total biliary obstruction, there is not only a general disturbance of the organism but a progressive injury to the liver leading to secondary cirrhosis.

Urobilinuria, however, fails to occur even though there is a marked bilirubinurea.

With local and partial biliary obstruction a moderate urobilinuria occurs but no bilirubinuria, due to the failure of the unobstructed hepatic tissue to remove urobilin from the blood as completely as bilirubin.

Elman, R., and McMaster, P. D.: Studies on Urobilin Physiology and Pathology. V. The Relation Between Urobilin and Conditions Involving Increased Red Cell Destruction. Jour. Exper. Med., November, 1925, xliii, No. 5, p. 619.

An experimental study conducted with dogs.

Much of the interest in urobilin arises from its appearance in the urine in diseases characterized by excessive destruction of red blood cells.

In accordance with the results of studies previously reported, urobilinuria does not occur when the entrance of bile into the intestinal canal is totally prevented, even when extensive blood destruction occurs.

When blood destruction occurs under conditions of partial bile loss, urobilinuria occurs.

Urobilinuria occurs during the extravasation of blood after operation but not during the absorption of extravasated blood from hematomas.

Excessive blood destruction during infections is associated with urobilinuria but its formation in the intestine is the essential factor.

Kirby-Smith, J. L., Dave, W. E., and White, G. F.: Creeping Eruption. Arch. Dermat. and Syph., February, 1926, xiii, No. 2, p. 137.

In a profusely illustrated article the authors describe the clinical symptomatology, appearance and treatment of a skin affection seen in Florida.

They have demonstrated by means of serial sections of tissues removed at biopsy that the disease is due to the entrance into the skin of a migratory nematode larva approximately $\frac{1}{50}$ inch in length and $\frac{1}{12,000}$ inch broad.

No definite conclusion can as yet be drawn regarding the host of the adult worm, but it is suggested that the rat may be the normal host and that poultry, cats, dogs, and other animals may be concerned.

MacCarty, W. C.: The Cancer Cell and Nature's Defensive Mechanism. Surg., Gynec. and Obst., December, 1925, 783.

MacCarty presents the observations leading to his belief that the cancer cell has distinct morphologic characteristics whereby it may be recognized.

These cells are described usually as they have been seen when dead for some time and after they have been subjected to the action of various reagents which produce cytologic changes.

MacCarty's observations were made upon living cells and upon fixed but not embedded cells by methods he has already described.

Cancer cells cannot always be distinguished from regenerative cells but this can be done frequently on the difference between the volume-relationship between nucleus and nucleolus and the whole cell, this being greater in the malignant cell.

The regenerative cell is more delicately constructed and its nuclear granules are finer.

Familiarity with the picture is essential to certainty of recognition. Cellular differentiation is an important factor in prognosis, but it is not the only factor.

The cancer cell in the living state is seen as an ovoid or spheroid body with no irregularity of "cell wall," nucleus, or nucleolus; the demarcations of the component parts of the cell are sharp and distinct; the granules are fairly uniform in size, and the mitotic figures are sometimes multipolar but not asymmetrical.

The article is clearly illustrated with line drawings and microphotographs, and the author's conception of the cancer cell is best understood by reference to and study of the illustrations.

Heidelberger, M., and Avery, O. T.: The Soluble Specific Substance of Pneumococcus. Jour. Exper. Med., July, 1923, xxxviii, No. 1, p. 73.

Heidelberger, M., and Avery, O. T.: The Soluble Specific Substance of Pneumococcus. Second Paper. Jour. Exper. Med., September, 1924, xl, No. 3, p. 301.

Avery, O. T., and Heidelberger, M.: Immunologic Relationships of Cell Constituents of Pneumococcus. Jour. Exper. Med., July, 1923, xxxviii, No. 1, p. 81. **Second Paper:** Jour. Exper. Med., September, 1925, xlii, No. 3, p. 367.

Heidelberger, M., Goebel, W. F., and Avery, O. T.: The Soluble Specific Substance of Pneumococcus, Third Paper. November, 1925, xlii, No. 5, p. 727.

Avery, O. T., and Morgan, H. J.: Immunological Reactions of the Isolated Carbohydrate and Protein of Pneumococcus. Jour. Exper. Med., September, 1925, xlii, No. 3, p. 347.

Avery, O. T., and Neill, J. M.: The Antigenic Properties of Solutions of Pneumococcus. Jour. Exper. Med., September, 1925, xlii, No. 3, p. 355.

Heidelberger, M., Goebel, W. F., and Avery, O. T.: The Soluble Specific Substance of a Strain of Friedlander's Bacillus. Jour. Exper. Med., November, 1925, xlii, No. 5, p. 701.

Part II: Chemical and Immunological Relationships of Pneumococcus Type II and a Strain of Friedlander's Bacillus. Jour. Exper. Med., November, 1925, xlii, No. 5, p. 701.

The papers, the titles of which appear above, form a record of observations during the past several years, the salient features of which may thus be summarized.

In 1917 Dochez and Avery showed that whenever pneumococci were grown in fluid media, there was present in the culture fluid a substance which was precipitated by homologous antipneumococcus serum. A similar or analogous substance was demonstrated in the blood serum, urine, and body fluids of animals and human beings infected with pneumococci.

It was found possible to isolate this substance by precipitation with alcohol, acetone, and ammonium sulphate and to demonstrate that it consisted of a carbohydrate which appeared to be a polysaccharide built up of glucose molecules.

In the course of the isolation of this specific carbohydrate substance an additional substance, precipitable in the cold by acetic acid and consisting of nucleoprotein and mucoid was encountered.

This protein fraction of the bacterial cell was not type specific but reacted with antipneumococcus serum regardless of type derivation, thus differing from the carbohydrate fraction.

Further studies developed a method of securing the carbohydrate substance in a more purified form. The details of this newer method are somewhat lengthy and must be found in the original paper (Jour. Exper. Med., September, 1924, xl, No. 3, p. 301).

This purified product contains less than 0.2 per cent of nitrogen and is identified as a polysaccharide.

Immunologic studies of these two components of the pneumococcus cell (Jour. Exper. Med., September, 1925, p. 367) have shown that the carbohydrate substance is the type-specific substance, being chemically distinct and serologically specific for each of the three fixed types. The protein fraction, on the other hand, is common to all the pneumococci and is species-specific but not type-specific.

The carbohydrate fraction is nonantigenic; injection of the protein fraction produces an immune serum reacting with pneumococcus protein in general. These antiprotein sera do

not agglutinate type-specific strains and do not precipitate solutions of the carbohydrate substance.

Morphologic integrity of the bacterial cell is hence necessary for the expression of its full antigenic power.

The elaboration of the specific carbohydrate substance is a specialized function related to the capsular development and is most marked under optimal growth conditions.

In a continuation of these studies the type-specificity of the carbohydrate substance was demonstrated, that from each of the three fixed types differing from the others chemically, but all possessing a definite immunologic relationship.

Intact pneumococci give rise to agglutinins of homologous type and to precipitins for their type-specific carbohydrate.

Solutions of pneumococci fail to stimulate the formation of type-specific antibodies due to changes occurring during the dissolution of the cell.

The antigenic properties of solutions of the isolated protein and carbohydrate substances have been noted above.

It is suggested that if final proof can be brought for the conception that the capsular zone of the organism is largely composed of a carbohydrate specific substance, is part of the defense mechanism of the cell, and the site of its initial contact with antibody, then these soluble polysaccharides acquire a new significance, not only in the serologic reactions of the cell, but in the actual processes of infection and immunity in the host.

Further studies (Jour. Exper. Med., November, 1925) demonstrated that the polysaccharide material isolated by Toenissen in 1901 from the capsular material of B. Friedlander was closely related to the specific carbohydrate secured from the pneumococcus and the principle sugar from which the specific substance of Friedlander's bacillus was built up.

This substance and a similar substance from Type III pneumococci were found to be reciprocally precipitable in each other's antiserum.

The strain of Friedlander's bacillus and the Type III pneumococcus were found to be agglutinable in each other's antiserum, this relationship, however, not extending to all members of the Friedlander group.

Agglutinin absorption was not reciprocal, only those for the absorbing organism being removed. While cross precipitin reactions occur the absorption of precipitins was not reciprocal.

Protection experiments in mice were reciprocal and heterogenetic specificity is thus evidenced.

It appears probable that when the analogous specific polysaccharides of unrelated microorganisms correspond in chemical constitution, an immunologic correspondence results and a new avenue for the study of bacteria as disease-producing agents is thus opened up.

The methods for the isolation of the specific substances discussed are given in detail but are too lengthy to be abstracted satisfactorily.

Lutz, A.: A Method for Transferring Tubercle Bacilli from Solid to Liquid Culture Media. Am. Rev. of Tuberc., November, 1925, xii, No. 3, p. 270.

Fifty c.c. of glycerin agar is placed in a 100 c.c. Erlenmeyer flask. After sterilization the medium is allowed to harden in a slant running from just below the mouth to the bottom of the slant. When the slant is hardened, 15 c.c. of sterile glycerin broth is added; the flask is then sealed with a paraffined stopper and stored in an upright position.

A heavy inoculation is made on the slant down to the fluid level. A better growth is obtained if the plug is not sealed in but placed in lightly for the first two days of incubation. The growth covers the solid surface and spreads out over the surface of the broth by continuity.

If the spread is poor a portion of the pellicle may be pushed off onto the broth. Once there is growth on the broth, transfer to other broth cultures is easy.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*The Biology of the Protozoa**

THE underlying principle in this presentation—the fruit of many years of research and teaching—is the irritability of protoplasm, combined with protoplasmic organization, the latter being specific in type.

This fundamental conception as applied to the protozoa is fully developed by the author for the purpose of developing a common point of view from which new observations and experiments may be evaluated.

While somewhat controversial at times, the monograph presents an analysis of a wealth of careful studies and observations, both those of the author and of protozoologists in general, and should be not only of interest but of great value to advanced students of this subject.

Insulin, Its Use in the Treatment of Diabetes†

THIS very excellent contribution is not only a critical summary of the insulin work done up to the present time and a presentation of our knowledge of its physiology and pharmacology but also and particularly it is a monograph on the treatment of diabetes, both with diet and with insulin. The first section, written by Dr. MacLeod, carries an historical résumé of the development of insulin and the presentation of our present knowledge of its action. From it we learn that there is much yet to be known of its mode of action. Insulin appears to accelerate the rate at which sugar passes into the tissues, but exactly what happens to the sugar after it has reached the tissues is uncertain. Apparently some compound is made with phosphorus. Evidently there is an interdependence between sugar and phosphorus in the body. In this connection it is of interest to note the work of Embeden, showing that a compound of phosphoric acid with a complex yielding lactic acid, undergoes decomposition as a result of muscular exercise.

The work of MacLeod and his associates on depancreatized dogs, which eventually die with symptoms of acute liver breakdown even though maintained on adequate insulin treatment but which are kept alive and apparently well for an indefinite period when the meat eaten is changed to raw pancreas, is most interesting. The small amount of active trypsin that ap-

*The Biology of the Protozoa. By G. N. Calkins, Ph.D., Professor of Protozoology, Columbia University. Pp. 623. 238 engravings. Cloth. Price $7.50. Lea and Febiger, Philadelphia.

†Medicine Monographs. Vol. VI. Insulin, Its Use in the Treatment of Diabetes. Part I. Physiology. By Dr. J. J. R. MacLeod. Part II. Clinical Section. By W. R. Campbell, M.D. Pp. 242. Cloth. Williams & Wilkins Co., Baltimore, 1925.

parently gets into the system following this method, evidently prevents the untoward end-results.

MacLeod reaches no clear-cut conclusions with regard to the action of insulin. By the time the second edition of the book goes to press he will have much to add. On the other hand, we venture to predict that little of what has been written will require changing.

The second and by far the larger portion of the book is devoted to the clinical section and is written by Dr. W. R. Campbell. It is the best exposition of the clinical action and use of insulin which we have as yet met. The procedures for regulating the diet and the diet tables are distinctly original and greatly simplified. They are such that even the physician who treats but an occasional diabetic can ill afford to dispense with the assistance which he will derive from a study of this contribution.

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*Lead Poisoning**

LEAD poisoning, now a relatively infrequent condition, but one which in the past has been of greatest importance, yet another of those diseases "first described by Hippocrates," is a clearly defined clinical entity, as a rule easily recognized. And yet our knowledge of the modus operandi of this inorganic poisoning is still quite obscure.

In years past lead poisoning occurred quite generally following the use of pewter dishes, the drinking of water which had passed through lead pipes, and the taking of lead medicinally. Even yet industrial lead poisoning is a factor to be taken into account. One of the great achievements of industrial medicine has been the elimination of much of this type of illness, but scarcely more than a year ago there was a veritable epidemic of industrial poisoning from tetraethyl lead.

Aub and his collaborators have studied the problem of lead poisoning from all angles and have collected their results into a comprehensive monograph. The chemical studies are particularly interesting. They find that lead exists in the blood in only a very small amount. Even in severe lead poisoning 2.8 mg. of lead per liter of blood is a high reading. The usual conception is that lead exists as an albuminate, but they conclude that in the blood it is present in colloidal suspension rather than in solution and in an inorganic form, namely, colloidal lead phosphate. They find that the only storage depot of importance is the bones, where lead is deposited again as the phosphate, replacing calcium.

Probably the most common source of absorption is through the intestinal tract, but absorption also definitely occurs through the respiratory tract, and when it occurs in this way, it produces more rapid and serious symptoms. In part this is probably due to the fact that the introduction is directly into the systemic circulation, while with gastrointestinal absorption the lead passes

*Medicine Monographs. Vol. VII. Lead Poisoning. By Joseph C. Aub, Lawrence T. Fairhall, A. S. Minot and Paul Reznikoff, with a chapter on the prevalence of industrial lead poisoning in the United States by Alice Hamilton. Pp. 265. Cloth. Williams & Wilkins, Baltimore, 1926.

through the liver where a good part of it is removed and excreted in the bile. The authors do not agree that lead cannot be absorbed through the skin but leave this open as a moot question. It is excreted through various channels, the chief of which is the gastrointestinal tract.

The authors find that the excretion of lead is controlled by essentially the same factors which govern calcium excretion. Magnesium, sodium, and potassium salts exert a decalcifying effect, dependent upon the fact that the organism tends to maintain a normal ratio between the concentration of these various salts in the body fluids. Better results in the removal of both calcium and lead, however, were obtained with acids, particularly those which cannot readily be burned in the organism but must be neutralized by the body bases. These are such as hydrochloric acid, either as such or as acid phosphate, and ammonium chloride, which on absorption produces hydrochloric acid. Alkali, on the other hand, tends to conserve calcium and lead, but if sufficient is given to produce an alkalosis, the tertiary lead phosphate is rendered more soluble, and so, here too, the excretion of lead is promoted.

Experimentally, even after the excretion of lead has been promoted for a long time, chemical examination of the bones shows considerable residual storage of the element. In its insoluble form in bone it apparently causes no trouble, and the authors raise the question as to whether it might not be better to favor complete storage of lead in the bones by high calcium intake and a careful maintenance of normal acid-base equilibrium.

Potassium iodide likewise favors excretion of lead, apparently chiefly because of the action of the iodine ion, but it is not as efficacious as the acids and acid-forming salts. The latter are more effective when the calcium intake is low.

The stippling of red blood cells, anemia, increased resistance to hypotonic saline and kindred phenomena appear to be dependent upon surface contact of the lead salt with the red cells. The so-called lead palsy is not a true neuritis but is dependent for its origin upon muscular damage and occurs more frequently in those muscles which are used more generally. Lead inhibits the motility of smooth muscle while it increases its tone.

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EDITORIALS

Dye Therapy

IN RECENT years a great deal of attention has been paid to the possible treatment of infection by the intravenous use of certain dyes, and some difficulty has been experienced in explaining satisfactorily the somewhat conflicting reports which have accumulated.

In any consideration of the subject it is necessary to distinguish between those dyes which act upon bacteria as dyes and whose effects are due to various radicals in their formula, and those whose action is due to bactericidal elements combined with or forming the dye.

Representative of the former group is gentian violet, a member of the triphenylmethane group of dyes, whose bacteriostatic and bactericidal activities are restricted to certain groups of bacteria and even, at times, to certain strains of a single group. Illustrative of the latter group is mercurochrome, a combination of a metal and a fluorescent dye, whose bactericidal effects are due to the mercury in the combination.

In view of these inherent and important differences, it is of advantage to consider them separately.

The use of gentian violet arose from the work of Churchman who demonstrated by the study of pure bacterial cultures that, when exposed to the action of this dye, four phases of bacterial inhibition might be observed: namely, (1) cessation of motility; (2) inhibition of reproduction; (3) suspension of bacterial activity for varying periods; and (4) inhibition of sporulation.

Of very great interest is the fact that, by varying the proportions of the dye, any or all of these forms of inhibition may be produced, and of still greater interest the further fact that, once released from the influence of the dye, the bacteria may resume all their normal and usual activities.

To express this power to inhibit bacterial activity the term *bacteriostosis* has been coined.

Churchman's studies have shown that gentian violet possesses bacteriostatic properties, that these are most marked for gram-positive organisms, and that the dye may even be bactericidal.

He has also shown that acid fuchsin has an action contrary to that of gentian violet, and that, by a mixture of dyes, the combined and complementary action of both may be obtained.[1]

A satisfactory explanation of the mechanism of these effects of gentian violet upon bacterial cultures remains to be found. Apparently, it is not so much a question of the penetration of the dye into the bacterial cell and possibly more closely related changes—perhaps a change in the P_H—effected by the dye at the surface of the organism.[2] In the case of acid fuchsin, the bacteriostatic effects have been ascribed tentatively to the presence of SO_3 radical in its formula.[3]

The therapeutic application of these studies was inevitable and very definite, and excellent results were obtained in the treatment of local infections (diphtheria carriers, etc.), and in the treatment by injection of infections in closed cavities, such as empyema and joint cavities.

From this to the treatment of conditions characterized by or associated with bacteriemia was but a step, and it is in this field that disconcerting discrepancies between *in vitro* and *in vivo* results have been encountered, the consideration of which is attracting deserved attention.

Chemotherapy has always been influenced by and associated with the ideas of Ehrlich and until recent times was dominated by two conceptions: of a therapia sterilans magna and a specific chemical affinity between the drug and the organism. These conceptions, however, have been modified necessarily by the observations of more recent times.

It is not to be denied that very definite, and at times even dramatically startling, results have been obtained after the intravenous administration of gentian violet and mercurochrome. On the other hand, it cannot be gainsaid that equally striking and contradictory failures have been reported.

Some of these contradictions may be explainable by lack of controlled

observations, unsupported assumptions, and, as Churchman has pointed out,[4] by a lack of definition as to exactly what constitutes a septicemia.

While the term includes and indicates a bacteriemia, differentiation must be made between transient bacteriemias, such as may occur in health, and bacteriemias associated with such diseases as pneumonia or typhoid fever, which are clinical and pathologic entities, and those conditions which, beginning as localized or focal infections, terminate in or are complicated by extensive, repeated, continuous, or massive bacterial invasion of the blood stream.

As indicated by the above terms, it is clear that *septicemia* may include a variety of conditions, the gravity of which is influenced by a variety of factors. Among these may be mentioned: (a) the virulence of the invading organism; (b) the accessibility of their source of origin—the focus of infection—to treatment; (c) the resistance of the patient; and (d) the site and gravity of the secondary lesions resulting from bacterial localization in various organs or tissues of the body.

It is fallacious, as Churchman comments, to assume that, in septicemia, bacteria travel round and round in the blood stream reproducing as they go, and that injected antibacterial substances travel with them. The bacteria originate in and enter the blood from some focus; they are removed from the blood by the defensive mechanism, which includes the mechanical filtration by lymph glands, etc., as well as specific antibodies, phagocytes, and the like.

It is obvious that there are many factors entering into and influencing the end-results of septicemia and influencing both the successes and failures which have been reported.

It can be stated that the beneficial effects reported are not to be explained upon the basis of a therapia sterilans magna nor as resulting entirely from a specific bactericidal effect of the dyé upon the organism while both are coursing through the blood stream.

Churchman and Herz,[5] for example, have shown that gentian violet, even when injected in massive doses, rapidly disappears from the blood, and Churchman has recorded his inability to kill organisms in the blood by the injection of gentian violet.

Brill and Myers[6] found the maximal advisable concentration of gentian violet or mercurochrome in the blood stream—1: 10,000—without bactericidal action upon staphylococci, streptococci, or the colon bacillus.

Meleney and Zan[7] were unable to demonstrate any bactericidal action of neutral acriflavine on hemolytic streptococci, even when injected in lethal doses in rabbits. Walker,[8] in an investigation of the bactericidal properties of freshly defibrinated blood to which mercurochrome was added in varying concentrations, found no increase in bactericidal activity toward the colon bacillus in concentration of mercurochrome as high as 1:400; with concentrations of 1:200 the bactericidal activity of the blood was destroyed.

Staphylococci and streptococci were found to grow more luxuriantly in the mercurochrome-blood mixture (1:400) than in plain blood, due, apparently, to the injurious action of mercurochrome on leucocytes.

Other and similar reports are to be found in the literature to confront and confuse the observer likewise confronted with clinical reports of definite and striking "cures" and recoveries.

An important angle of the question, which has only recently begun to receive the attention it deserves, is the fact that chemotherapy, as Kolmer has said, may be associated with chemopathology.

Young, Scott and Hill[9] have reported that 900 mg. of mercurochrome may be administered daily by mouth for ten days without injury, and Hill and Bidgood[10] state that definite renal damage does not follow intravenous administration of mercurochrome unless the dose is at least 10 mg. per kg.; the slight damage done by smaller amounts is not irreparable.

St. George,[11] however, in necropses of five cases treated with mercurochrome, found in all intense nephritis and intense congestion of the cecum and superficial colonic ulcerations characteristic of mercurial poisoning, mercury being recovered from the viscera in amounts larger than those seen in bichloride poisoning. The largest amount of mercurochrome administered was 0.9 gm. (90 c.c. of 1 per cent solution) the smallest, 0.1 gm. (20 c.c. of 0.5 per cent solution).

Corper[12] has shown that the intracutaneous injection of 0.2 c.c. of 0.5 per cent mercurochrome produces ulceration; that intratracheal injection produces distinct pathologic changes persisting for as long as four days, the severity varying with the amount and concentration of the solution; and that intrapleural injection may produce permanent parenchymal damage.

It is apparent, therefore, that these agents not only cannot be expected to produce a therapia sterilans magna, but that they must be used with care and due regard for the cumulative and even dangerous chemopathology which may result.

The mechanism of the clinical results observed still remains to be explained.

In the case of gentian violet, Burke and Hewton[13] have shown that the intensity of the bacteriostatic and bactericidal activity of this dye may be influenced by variations in the character of the solutions; the more alkaline the solution, the greater the bactericidal activity, and vice versa. As a stable alkaline solution cannot be prepared, they suggest the use of a buffered solution near neutrality or a *freshly prepared* solution in 3 per cent sodium bicarbonate.

It is not known just how dyes produce cellular death nor what is the relation of alkalinity to this reaction. Burke and Grieve,[14] however, have shown that the bacteriostatic and bactericidal activity of many dyes varies with the P_H of the medium surrounding the organisms and that, within certain limits, alkalinization of the body fluids may augment the action of the dye.

In the face of the discordant data, the contradictory character of which has been indicated, Churchman[4] has summed up the possible explanations of the results on record as follows:

Admitting that the dyes can be introduced into the circulation only in relatively small amounts, that they are rapidly removed as such, and that it is hardly conceivable that they remain in contact with bacteria for a time sufficient to permit specific antibacterial action, there still remains the possibility that they may be changed in the body into substances more potent than the mother dye. Or, again, it is possible that they may stimulate the production of antibacterial substances in the tissues.

Still further, as both bacteria and dye stuff are removed from the blood stream by various agencies, it is possible that in various organs or foci a more prolonged and intimate contact of bacteria and dye may occur and so bactericidal or bacteriostatic reactions may be encouraged.

Of very great importance, perhaps, is the *bacteriostatic* activity of dyes as opposed to or contrasted with their bactericidal powers, by means of which the bacteria are inhibited, their growth and activities held in abeyance; opportunity is thus afforded for the various defensive mechanisms of the body to be mobilized to come into play.

A still further theory is that the activity of toxins may be affected or restrained by dyes, in the case of fluorescent compounds, such as mercurochrome, perhaps, by some photodynamic action. This, however, is entirely speculative and remains to be demonstrated.

All that can be said so far is that a therapia sterilans magna cannot be achieved by the use of dyes and, indeed, there are factors, such as the possible sudden liberation of massive doses of endotoxin, which render this not always desirable.

The bacteriostatic powers of both gentian violet and mercurochrome appear to be of more therapeutic importance than their bactericidal abilities, and the ultimate results consequent upon their use depend in no small measure upon the resisting powers of the patient to which, in all probability, they bring increased opportunity for mobilization and attack upon the invading bacteria, temporarily inhibited by the bacteriostatic effect of dye therapy.

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—*R. A. K.*